JUN 17 1946

# JOURNAL OF ANATOMY

## ORIGINALLY THE JOURNAL OF ANATOMY AND PHYSIOLOGY

CONDUCTED ON BEHALF OF THE ANATOMICAL SOCIETY OF GREAT BRITAIN AND IRELAND BY

J. C. BRASH
J. D. BOYD
A. J. E. CAVE
W. E. LE GROS CLARK
F. GOLDBY
W. J. HAMILTON
J. KIRK (TREASURER)
C. M. WEST (ACTING EDITOR)

VOLUME 80

PART 2

APRIL 1946

*Entered at New York Post Office as Second Class Matter*

*(All rights reserved)*

CAMBRIDGE UNIVERSITY PRESS

LONDON: BENTLEY HOUSE

CHICAGO: The University of Chicago Press
(Agents for the United States)
BOMBAY, CALCUTTA, MADRAS: Macmillan

*All rights reserved*

*Price Twelve Shillings and Sixpence net*

PRINTED IN GREAT BRITAIN

**Books** *ON*

*ANATOMY AND MEDICAL SCIENCES*

of all publishers
supplied from stock.

*LENDING LIBRARY*

*MEDICAL & SCIENTIFIC*

NEW BOOKS AND LATEST EDITIONS ALWAYS OBTAINABLE

ANNUAL SUBSCRIPTION, TOWN OR COUNTRY, FROM ONE GUINEA

*DETAILED PROSPECTUS ON APPLICATION*

**H.K. LEWIS & Co. Ltd.**
136 Gower St., LONDON, W.C. 1
Telephone: EUSton 4282 (5 lines)

Large Department for
MEDICAL BOOKS

**FOYLES**

LARGEST BOOKSHOP IN THE WORLD

- Over 3,000,000 books in stock
- New and second-hand books on every subject
- Catalogues free
- Any book sent on approval

BOOKS BOUGHT

119-125 CHARING CROSS ROAD
LONDON, W.C. 2
Tel. GERrard 5660 (16 lines)
Telegrams: Foylibra, Westcent, London
OPEN DAILY 9-6, including Saturdays


## RESEARCH BOARD FOR THE CORRELATION OF MEDICAL SCIENCE AND PHYSICAL EDUCATION

### *Research Prize*

The Research Board has decided to offer an Annual Prize in order to encourage scientific investigation into the basic principles of Physical Welfare.

A First prize of £200, a Second prize of £50, will be offered in 1947 for a Thesis embodying Original Research on some aspect of the following field of inquiry:

"The Range of Movement at Joints, considered with reference to normal function and to disability,

*Either* In the Joints of the Foot,

*Or* In the Joints of the Vertebral Column."

Entrants for this research prize should be either of British nationality or ordinarily resident in the British Empire.

Five typewritten copies of the Thesis should be submitted to the Research Board by *September 30th, 1947.*

To ensure that the thesis falls within the relevant field of enquiry, candidates should previously submit for the approval of the Research Board a statement of the nature of their proposed investigation.

Further information and suggestions can be obtained from the CHAIRMAN OF THE RESEARCH BOARD FOR THE CORRELATION OF MEDICAL SCIENCE AND PHYSICAL EDUCATION, 87, HARLEY STREET, LONDON, W. 1.

# REPAIR OF FRACTURES OF THE PARIETAL BONE IN RATS

By J. J. PRITCHARD, *Anatomy Department, St Mary's Hospital Medical School, London*

It is well known clinically that fractures of the cranial vault frequently are closed by fibrous union, and that new bone is produced in smaller amount than after comparable injuries to long bones. No complete explanation of these facts has been given. It therefore seemed desirable to study the healing of simple linear fractures of the cranial vault, experimentally produced, with the object of determining the factors responsible for both the deficiency of new bone formation and the failure of bony union.

## MATERIAL AND METHODS

The Lister strain of black-and-white hooded Norwegian rats was used in the experiments, which formed two series. In series A the rats were from 12 to 18 months old and the left parietal bone was cut with an electric circular saw; in series B they were from 6 to 12 months old and the left parietal bone was incised with a scalpel. All operations were performed aseptically under ether anaesthesia. The shaved scalp was cut in the middle line and retracted to expose the left parietal bone, in which a sagittal cut was made through pericranium, bone and dura mater, approximately 2 mm. medial to the temporal line, and about 15 mm. in length.

The animals were killed at intervals varying from 0 to 120 days after operation. Their skulls, dissected free from skin, were fixed entire in Zenker's or Bouin's fluid, and were later embedded in celloidin or paraffin. The celloidin blocks were cut at 25 $\mu$ and the paraffin at 5 $\mu$. From 6 to 12 sections from different parts of the fractured bone were mounted, and stained with Weigert's iron-haematoxylin and Van Giesen or with Ehrlich's haematoxylin and eosin.

Thirty animals were operated upon, and the results from 23 of them are described below.

## DESCRIPTION OF EXPERIMENTS

It should be noted that the rat's parietal bone is about 0·25 mm. thick and devoid of diploë in the centre, but near the margins it increases to 0·35 mm. in thickness and contains a diploic labyrinth. The experimental fractures involved both parts of the bone and thus the appearance of the sections varied according to the parts of the block from which they were taken.

The width of the fracture gap in series A (saw-cuts) was approximately 1 mm. but in series B (knife-cuts) only 0·1–0·5 mm. It was found possible to measure the width of the original gap in most specimens many weeks after fracture because no remodelling of the bone took place during the period of the experiments, and because new bone was readily distinguished from old long after healing. The new bone was at first reticular, but later on reticular and lamellar elements were mingled, while the old bone was wholly lamellar. The new bone differed also in its staining reactions, having less affinity for picric acid and more for Ehrlich's haematoxylin than the old bone.

In order to simplify the account of the repair process the animals have been divided into four groups:

(*a*) Rats 1–10 show the sequence of events after linear saw-cuts, uncomplicated by infection or excessive haemorrhage.

(*b*) Rats 11–14 show the repair of saw-cuts complicated by widespread inflammation attributable to infection or haemorrhage.

(*c*) Rats 15–21 show repair of linear knife-cuts without complications.

(*d*) Rats 22 and 23 show repair of linear knife-cuts with similar complications to (*b*).

### *Group (a)*

*Rat* 1. *Killed immediately after operation.* This specimen showed the type of lesion produced by the saw (Pl. 1, fig. 1). A linear gap, approximately 1 mm. wide and filled with fresh blood clot, had been produced in the left parietal bone, dividing it into medial and lateral fragments. The saw had cut the outer layers of the bone cleanly, but the inner part was torn and frayed so that long spicules were depressed into the surface of the brain. The dura mater was completely severed. On the lateral fragment the pericranium remained intact as far as the cut edge, but it was stripped from the medial fragment for 0·5 mm. from the fracture. The dura mater was more extensively detached and was separated from the bone by blood clot for a distance of 2 mm. on each side of the cut.

It is probable that the initial lesions were similar in rats 1–14, because all the operations were performed with the same saw in the same manner, and because depressed spicules and detached dura mater were observed, as in this specimen, in all the sections examined, despite the changes effected by the organization of blood clot and the deposition of new bone in the course of repair.

*Rat* 2. 2 *days' survival.* In this specimen the blood clot between the fragments and above the detached dura mater showed early signs of organization: polymorphonuclear leucocytes and fusiform cells were present in small numbers among the strands of fibrin. It was not possible at this stage to distinguish fibroblasts from osteoblasts within the blood clot, though the latter cells were readily identified on both the outer and inner surfaces of the skull where the periosteum remained intact. Osteoblasts were active within the diploë also, where it was opened by the saw. In all the specimens of group (*a*) these reactions were found only in the immediate neighbourhood of the fracture, the skull and connective tissue farther than 2 mm. from the gap being quiescent in all stages.

*Rat* 3. 4 *days' survival.* The blood clot showed more advanced organization, with the appearance of fusiform cells, mononuclear phagocytes, new capillaries and a few polymorphs. On both surfaces of the skull, where the periosteum had not been detached, proliferating osteoblasts were found surrounding slender trabeculae of new bone.

*Rat* 4. 8 *days' survival.* The blood clot had now been resorbed and in its place loose vascular connective tissue had made its appearance between the fragments and the continuity of the pericranium was restored by fibrous tissue which bridged the gap. Above the detached dura mater a wedge-shaped zone of reticular new bone replaced the blood clot formerly present. Beneath the pericranium there was a very thin (0·04 mm.) layer of new bone which projected into the gap for 0·25 mm. and extended about 1 mm. from it on each side.

*Rat* 5. 16 *days' survival.* The gap in the bone was now filled with mature fibrous tissue containing a few multi-nucleated giant cells surrounding particles of bone dust, but nowhere had bony union been established. On the inner surface of the skull new bone filled the wedge-shaped space above the detached dura mater as in the previous specimen and extended along surviving spicules into the fracture gap for a short distance. On the outer surface only a single lamella of new bone had been added (Pl. 1, fig. 2).

*Rats* 6–10. *Survival periods of* 27, 35, 49, 70 *and* 77 *days respectively.* All these specimens showed similar features and none showed any advance on the 16-day specimen. The gaps were filled with mature fibrous tissue continuous with the pericranium and dura mater on each side. Bony union was not present in any specimen. New bone was found in the same situations and in the same quantity as in previous animals; it was consolidated, but still showed the initial reticular pattern among the superimposed lamellae. A surprising feature was the absence of any sign that bone had been resorbed in the course of remodelling, as is usual in the later stages of repair in long bones. Comparison of the stages of healing reached in the various specimens of this group made it evident that the production of new bone and fibrous tissue ceased between the 16th and 27th days after fracture.

*Group (b)*

*Rat* 11. 7 *days' survival.* In this specimen the fracture gap, 1 mm. wide, was filled with organizing blood clot, but the extra-dural blood clot remained unorganized. Above the fracture and extending outside the pericranium over both parietal bones was a thick layer of inflammatory exudate consisting of fibroblasts, new capillaries and mononuclear phagocytes, within a fibrinous network. This exudate was thickest immediately above the fracture where it contained a small focus of suppuration. The outer table of the medial fragment of the left parietal showed osteoclastic excavations, some of which penetrated through to the diploic spaces, in which some new bone was found. On the outer surface of the lateral fragment of the left parietal bone, and of the entire right parietal as far as the temporal line, a layer of reticular new bone had been formed reaching a maximum thickness of 0·1 mm. This new bone was interrupted by the sutural membranes, and ceased abruptly at the temporal lines. On the inner surface of the skull a very thin layer of new bone had been formed just beyond the stripped dura mater.

*Rat* 12. 11 *days' survival* (Pl. 2, figs. 5, 6). The fracture gap was filled with inflammatory cells continuous outside the pericranium with widespread exudate as in the previous specimen. Beneath the skull vault the blood clot had been replaced by reticular new bone. The outer table on each side of the fracture showed marked osteoclastic resorption and the diploë was exposed, but farther away new bone was present as far as the temporal lines, as in the last specimen. At this stage, however, the reticular bone was partly consolidated by the addition of lamellar bone.

*Rat* 13. 20 *days' survival.* The fracture gap was 2 mm. wide, twice its original width, a result of bone resorption which involved the medial fragment chiefly. Several small foci of suppuration were present within the gap, and inflammatory exudate extended across both parietal bones. New bone was present beneath the lateral fragment and within the diploë of the medial fragment; it covered the right parietal as well as the surviving parts of the left bone as far as the temporal lines, but was interrupted by the sutural membranes.

*Rat* 14. 77 *days' survival.* The bony gap was now 3 mm. and was filled with mature fibrous tissue. Destruction of bone had been greatest on the medial side of the fracture, as in the previous specimen. Consolidated new bone was readily identified over both parietals as before. There was at this stage no

active inflammation, but from the widespread distribution of new bone and the width of the bony gap, it was inferred that stages had been passed through comparable to those of rats 11, 12 *and* 13, in which widespread inflammation was present.

*Group* (*c*)

In this group the knife produced gaps in the left parietal bone between 0·1 and 0·5 mm. wide compared with 1 mm. for the saw cuts in groups (*a*) and (*b*). The knife also caused little destruction of bone and did not strip the pericranium and dura mater from the bone, although both these membranes were severed at the fracture line.

*Rat* 15. 7 *days' survival.* The gap was less than 0·5 mm. wide and most of the blood clot had been replaced by granulation tissue. For a distance of 1 mm. on each side of the fracture a single lamella of new bone, 0·02 mm. thick, had been deposited on the outer and inner surfaces of the skull. Where the diploë had been opened a similar thin layer was found.

*Rats* 16 *and* 17. *Both surviving* 10 *days.* These two specimens were similar in most respects; in both the blood clot had been entirely replaced by young connective tissue, and new bone formation was slight and limited to the same regions as in rat 15. Rat 16, however, showed a feature unique among the twenty-three animals studied, for in one place a small nodule of hypertrophic cartilage had been formed beneath the repaired pericranium of the fracture gap (Pl. 1, fig. 3).

*Rat* 18. 24 *days' survival.* The fracture was very narrow indeed, and in some places where the diploë had been opened the outer and inner tables were interlocked. Union was nevertheless by fibrous tissue except at one place where a fragment of old bone lay obliquely across the fracture gap and acted as a 'stepping stone' for new bone growing in from each side (Pl. 1, fig. 4). Beyond the fracture, for not more than 1 mm., a scanty layer of new bone had been deposited on the outer and inner surfaces and within the diploë as in previous specimens of this group.

*Rats* 19, 20 *and* 21. *Survival periods of* 60, 60 *and* 120 *days respectively.* The majority of sections from these three specimens showed established fibrous union with new bone serving merely to round off the edges of the fracture, but in a few places bony bridges united the fragments. New bone was not found more than 2 mm. from the edges of the fracture. There was no evidence of remodelling.

*Group* (*d*)

*Rat* 22. 6 *days' survival.* An inflammatory reaction of moderate severity was present over the whole of the left parietal area, and new bone covered the entire left bone and parts of neighbouring bones. On the inner surface and within the diploë, however, new bone was limited to the immediate vicinity of the fracture. In one place the gap between the fragments was very narrow and had been bridged already with new bone; elsewhere the gap was filled with organizing blood clot. Osteoclastic resorption was not seen.

*Rat* 23. 16 *days' survival.* During post-mortem dissection the scalp was found to be adherent to the fracture line over a small area and sections showed a small abscess surrounding fragments of old bone, and a gap in the parietal nearly 3 mm. wide; elsewhere the bone had not been resorbed and the gap was much narrower. Above the pericranium inflammatory exudate covered the entire left parietal and part of the right; beneath it new bone, reaching a maximum thickness of 0·2 mm., had been formed as far as the temporal lines. Inside the skull and within the diploë, new bone was present only near the fracture. In most sections new bone had spread into the fracture gap from each side, but only in a few places was the latter completely bridged; a narrow zone of fibrous tissue intervened between the fragments in most places (Pl. 2, fig. 7).

## CONCLUSIONS

*Group* (*a*). In these animals inflammatory reaction was localized to the fracture site. Blood clot between the fragments was rapidly organized and by the 8th day had been replaced by young connective tissue: established fibrous union was present in animals from the 16th day onward. Osteoblasts began to proliferate on outer and inner surfaces of the parietal bone after 2 days, and after 4 days reticular new bone appeared: this was at first confined to regions near the fracture where the pericranium and dura mater had not been detached, but it soon spread to replace extra-dural blood clot and to bury spicules of bone projecting into the fracture gap. Diploic and sub-pericranial new bone were very scanty and confined to a strip 1–2 mm. wide on either side of the fracture. Reticular new bone was later consolidated by lamellar bone; there was no remodelling.

*Group* (*b*). Widespread inflammation, with foci of suppuration near the fracture, characterized these specimens. Bone resorption was marked and the fracture was widened thereby. Healing was delayed until the inflammation had subsided, the gap was eventually filled by dense fibrous tissue. New bone formation was widespread beneath the pericranium, and both parietal bones were covered as far as the temporal lines; the new bone was interrupted by the sutural membranes. Diploic and extra-dural new bone were confined to the fracture site as in the previous group.

*Group* (*c*). These specimens showed only local

inflammation and very little new bone as in *Group* (*a*). In general, repair was by fibrous tissue, but in a few places bony union occurred where the gap was very narrow. In one specimen (rat 16) a small nodule of hypertrophic cartilage was found on one side of the fracture gap.

*Group* (*d*). These showed widespread inflammation and extensive sub-pericranial new bone formation as in *Group* (*b*). Bone resorption was marked in the immediate vicinity of foci of suppuration, but farther away the gap remained narrow and bony bridges were formed in a few places.

## DISCUSSION

It is clear that in these experiments the course of fracture repair is strongly influenced by the amount of inflammatory reaction. Inflammation is, of course, never entirely absent, but when the operation is accompanied by the minimum of haemorrhage and the wound heals aseptically, it is limited to the neighbourhood of the fracture and subsides within 10 days. In such cases (the majority of the series) new bone production is limited to the same region and is small in amount.

When, as a result of infection or excessive haemorrhage, there is widespread and intense inflammation in the loose connective tissue above the pericranium, new bone formation is more abundant and is approximately co-extensive with the inflamed area. This relationship between the degree and extent of inflammatory reaction, and the degree and extent of new bone formation, has often been observed in the past, and is not specific for the skull. It raises the general question whether osteogenesis is initiated by the same stimulus which causes the inflammation, such as the products of damaged tissue, blood clot or micro-organisms, or whether it is a result of a change in mechanical stress caused, for example, by the pressure of the exudate. Bone is known to react to altered mechanical stress, particularly during the growing period and on the other hand bone growth is markedly stimulated by suppurative conditions such as osteomyelitis. Though not designed to answer the question of the relative importance of mechanical and humoral factors in the stimulation of new bone formation, these experiments throw some light on it.

The new bone formed in relation to extensive inflammation was almost entirely on the outer surface of the skull and it ceased abruptly at the attachment of a thick fibrous membrane such as the temporal fascia (through which the inflammation did not extend). It seems unlikely that mechanical stresses, whether caused by the inflammatory exudate or otherwise, would be limited in this way; they would almost certainly affect the diploic and intra-cranial osteoblasts as well, and would not necessarily be limited by the temporal fascia. These experiments therefore support the contention that an important if not the main stimulus to osteoblastic activity in the case of the skull vault is humoral rather than mechanical. The absence of remodelling also suggests that mechanical forces are ineffective in the skull.

There remains for consideration the fact of complete or partial failure of bony union in these and other fractures of the cranial vault. A large number of factors could be listed which in one way or another promote bony union after fracture, but for the present discussion the following appear chiefly relevant:

(i) Close apposition of the fragments, or bridges across the fracture gap formed by bone fragments or grafts; (ii) the presence of unbroken periosteum joining the fragments; (iii) the absence of intervening fibrous tissue; (iv) the presence of cartilaginous or fibro-cartilaginous 'callus' across the gap, later to be replaced by endochondral ossification. The experimental findings can be discussed under these four headings.

Under (i) it may be said that intimate apposition of the fragments was not obtained because of the rigidity of the skull, whereas in long bones the fragments are held tightly together in most cases by muscular action. When the gap was only 0·1 mm. wide, as in the fractures made with a knife, bony union occurred in a few places, but never in the wider gaps (1 mm.) made with the saw. Even in the narrow gaps, such bony union as did occur was associated with the presence of fragments of bone comparable to grafts.

Condition (ii) obtains in many clinical fractures of long bones and its importance is shown by the fact that the first new bone to bridge the fracture gap follows the line of the intact periosteum. Heine (1837) and Troitzky (1932) found that linear fractures and even wide deficiencies of the skull were eventually repaired by bone if the dura mater was undamaged, but not otherwise. In the experiments described here the dura mater was always severed and fibrous union was the rule. It seems likely, therefore, that severing the dura mater is a major cause of the failure of bony union: possible reasons for this are considered below.

(iii) If a gap is made in a bone it will in general be filled either by new bone formed by local or migrating osteoblasts, by fibro-cartilaginous callus, or by fibrous scar tissue. In these experiments the blood clot within the fracture gap was replaced early by young connective tissue, and by the time osteoblasts began to migrate in from the sides a fibrous tissue barrier had been erected. On the other hand, the blood clot between detached dura and skull was not invaded by fibroblasts but was always rapidly and successfully replaced by bone. It appears, therefore, that osteoblasts would have

crossed the gap and laid the foundation of eventual bony union if fibroblasts had been prevented from organizing the blood clot prematurely. If this analysis is correct then intact dura mater should promote bony union in two ways: (1) by providing a layer of osteoblasts across the gap and so reducing the extent of migration necessary, and (2) by preventing the premature ingress of fibroblasts to the blood clot. It may be noted that both osteogenetic and 'limiting' functions for the dura are invoked by this explanation. Of course, if bone is produced by direct transformation of connective tissue cells without the aid of osteoblasts, as Leriche & Policard (1928) believe, then the division of the dura mater would be irrelevant to the question of bony union, but these experiments afford no support for their hypothesis.

(iv) In fractures of long bones fibro-cartilaginous callus usually fills the gap between the fragments at an early stage of repair (Urist & McLean, 1941). Cartilage is formed rarely in fractures of the cranial vault, and then only to a limited extent (cf. rat 16). This may be an inherent characteristic of bones like the parietal, but as the causes underlying cartilage formation in the course of fracture repair are not understood this cannot be stated with certainty. Hey Groves (1913) attributes its production to the effects of pressure and movement between the fragments, and it may be that these are absent in fractures of the rigid skull, where there is lack of apposition between the fragments.

It appears then that fibrous rather than bony union is common in fractures of the cranial vault mainly on account of the type of fracture (complete severance of dura mater and lack of apposition of the fragments) and of the peculiar conditions which obtain in the skull, such as the relative immobility of its parts. When close apposition is obtained and the dura is not divided bony union can be expected. It is doubtful whether there is any primary deficiency of osteogenetic power in the skull vault differentiating it from the rest of the skeleton, but it may have less capacity for forming cartilaginous 'callus', as might be expected on developmental grounds. The causes underlying cartilage production in fracture repair in general need further study before the poor development of cartilage in fractures of the skull can be explained.

## SUMMARY

1. Linear fractures were made with scalpel and saw in the left parietal bones of twenty-three adult rats, and the course of repair followed for 4 months.

2. Fibrous union was the usual outcome, but in a few cases bony bridges were formed across the fracture gap where this was very narrow and where fragments of bone acted as grafts.

3. New bone production was slight in amount and confined to the fracture site when healing was uncomplicated by widespread haemorrhage or infection. It occurred chiefly on the dural surface.

4. Widespread sub-pericranial new bone formation, with widening of the fracture gap by bone resorption, was found when healing was complicated by the effects of haemorrhage or infection.

5. Cartilage was found in only one specimen.

6. No remodelling took place in the course of the healing process.

7. Failure of bony union was attributed to the rigidity of the skull, lack of apposition between the fragments, the division of the dura mater and absence of fibro-cartilaginous 'callus'.

8. The evidence suggests that new bone formation is initiated by humoral rather than mechanical stimuli in the case of the skull vault.

I wish to thank Messrs A. V. Price and R. McKenzie Pratt for help with the animal operations, and Mr J. A. J. Fozzard for the photomicrographs.

## REFERENCES

Groves, E. W. Hey (1913). *Brit. J. Surg.* **1**, 438.

Heine, B. (1837). Reprinted in *Versuche ueber Knochenregeneration* (*Dtsch. Ges. Chir.*), 1926. Berlin.

Leriche, R. & Policard, A. (1928). *The Normal and Pathological Physiology of Bone* (trans.). London.

Troitzky, W. (1932). *Z. Morph. Anthr.* **30**, 504.

Urist, M. R. & McLean, F. C. (1941). *J. Bone Jt Surg.* **23**, 1.

## EXPLANATION OF PLATES

### PLATE 1

Fig. 1. Rat 1. 0 day survival. Shows characteristics of the lesion produced by the saw, before healing has begun. Note the blood clot (*BC.*) between the fragments, depressed spicules (*S.*), and detached dura mater (*D.*). ×67.

Fig. 2. Rat 5. 16 days' survival. Shows healing of a saw-cut fracture uncomplicated by infection or other cause of widespread inflammation. Note the scanty sub-pericranial new bone (*SPNB.*) and the relatively abundant extra-dural new bone (*EDNB.*), presumably replacing previous blood clot adjacent to the fracture. The fracture gap is now filled with fibrous tissue (*FT.*). ×112.

Fig. 3. Rat 16. 10 days' survival. Knife-cut. Shows a nodule of hypertrophic cartilage (*C.*) projecting from the edge of the outer table (*OT.*) and lying adjacent to sub-pericranial new bone (*SPNB.*). ×420.

Fig. 4. Rat 18. 24 days' survival. Knife-cut. Shows sub-pericranial new bone (*SPNB.*), diploic (*DNB.*) and extra-dural new bone (*EDNB.*) bridging a fracture gap with the aid of a detached fragment of old bone (*BF.*). ×112.

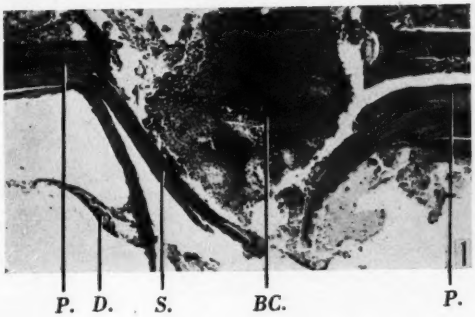
### PLATE 2

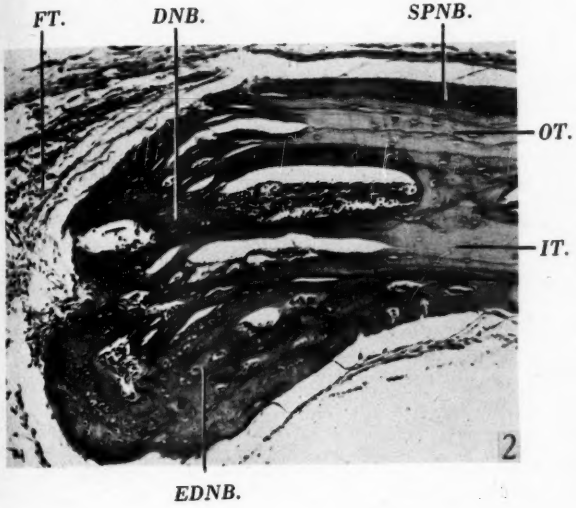
Fig. 5. Rat 12. 11 days' survival. Saw-cut. Shows a stage in healing of a fracture complicated by widespread inflammation. Note resorption of the outer table near the fracture (*ROT.*) but new bone formation beneath the inner table (*EDNB.*) Note also the spread of new bone along the spicule (*S.*). ×112.

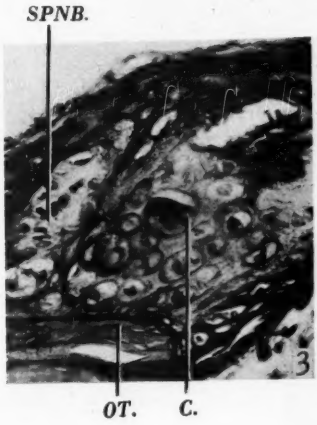
Fig. 6. Rat 12. 11 days' survival. Saw-cut. Shows a thick layer of sub-pericranial new bone (*SPNB.*) on the unoperated right parietal bone. There is no new bone within the diploë or on the inner surface. ×112.

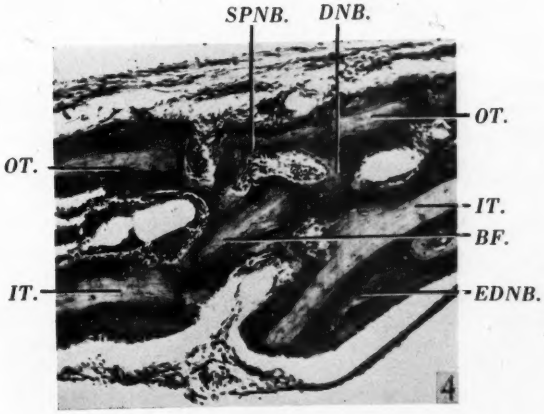
Fig. 7. Rat 23. 16 days' survival. Knife-cut. Shows abundant sub-pericranial (*SPNB.*) and diploic (*DNB.*) new bone in a fracture complicated by widespread inflammation around a focus of suppuration (involving another part of the fracture line from that illustrated). A thin layer of fibrous tissue (*FT.*) separates the new bone on either side. Extra-dural new bone (*EDNB.*) is relatively scanty. ×112.

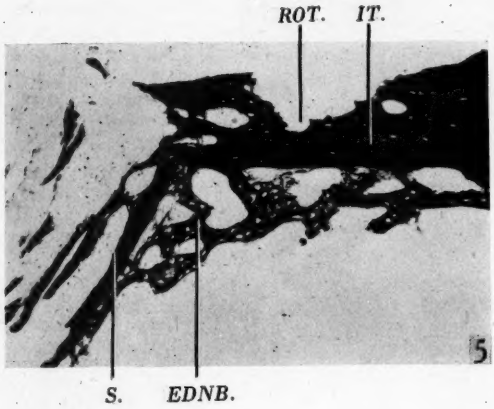
| | | | |
|---|---|---|---|
| *BC.* | Blood clot. | *FT.* | Fibrous tissue. |
| *BF.* | Bone fragment. | *IT.* | Inner table. |
| *C.* | Cartilage. | *OT.* | Outer table. |
| *D.* | Dura mater. | *P.* | Parietal bone. |
| *DNB.* | Diploic new bone. | *ROT.* | Resorbing outer table. |
| *EDNB.* | Extra-dural new bone. | *S.* | Spicule of old bone. |
| *SPNB.* | Sub-pericranial new bone. | | |

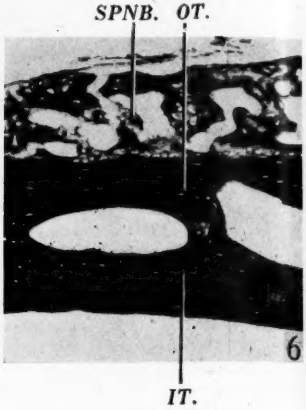

P.
D.
S.
BC.
P.
1

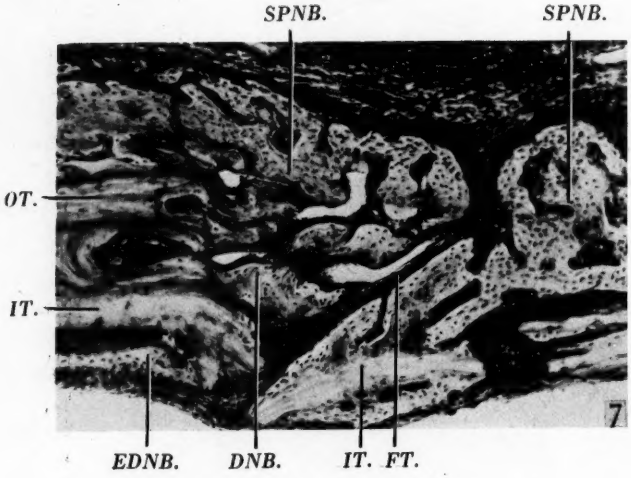

FT.
DNB.
SPNB.
OT.
IT.
EDNB.
2


SPNB.
OT.
C.
3


SPNB.
DNB.
OT.
OT.
IT.
BF.
IT.
EDNB.
4


ROT.
IT.
S.
EDNB.
5


SPNB.
OT.
IT.
6


SPNB.
SPNB.
OT.
IT.
EDNB.
DNB.
IT.
FT.
7

# MIGRATION PROCESSES DURING ONTOGENY WITH REFERENCE TO THE VENOUS DEVELOPMENT IN THE DORSAL BODY WALL

By J. AUËR, *Department of Anatomy, University of Utrecht*

## INTRODUCTION

During the early months of development many organs migrate from their original positions. Such migration may have a considerable effect on the topographical relations of adjacent structures. The present paper gives an account of the migration of the permanent kidney, the Wolffian body and the suprarenal gland, and of the possible effects of this on the development of the veins in the dorsal body wall.

The migration of the permanent kidney has for many years constituted an interesting problem which is not yet completely solved. The question naturally arises whether the migration of the permanent kidney is an active process on the part of the kidney, or is merely passive and the result of outside dynamic factors.

Recently Brockmann (1936) has attempted to summarize all the dynamic factors, and has drawn attention in particular to (1) the straightening of the body of the embryo, (2) the changes in the ventro-dorsal and transverse diameters of the body, and (3) the displacement of the centre of gravity. He is of the opinion that, between the stages of 10 and 20 mm., the straightening of the body is the principal factor. He thinks of the body as consisting of two layers *A* and *B* (Fig. 1). He considers the two layers as being fixed at the cranial end, and thus when the straightening of the body occurs the organs in layer *A* must move cranially relative to layer *B*.

Objections to Brockmann's theory have been made by Starkenstein (1938) on the grounds that (*a*) the embryos used by Brockmann were too far advanced for proper study of the migrating kidney, and (*b*) migration can be shown to be an active process, though he does not elaborate this. I believe that Brockmann's theory is too mechanical, and it does not explain the caudal migration of such organs as the heart and the testis. Brockmann perhaps recognized this, for he states (p. 627): 'Die Organe, die dem innerem Bande verglichen werden können, sind Blase, Enddarm, Ureter, Wolffscher Gang, Nieren, Urnieren und Aorta. Diese Organe werden bei der Entwicklung des Fötus in kranialer Richtung gegen die Wirbelsäule verschoben', thus omitting the testis. With regard to the Wolffian bodies and aorta he is certainly incorrect, as will be shown. I agree with Starkenstein that Brockmann's embryonic stages were too old. His youngest embryo measured 10 mm. and showed the kidney primordia at the level of lumbar vertebrae 3–5. Starkenstein found the primordia in younger embryos (3·6 mm.) in the region of sacral vertebrae 2–5, a conclusion quite in accordance with my own observations.

Little attention has been paid to the relation between migration processes and venous development in the dorsal body wall. The permanent kidney, the suprarenal gland, the Wolffian body and the visceral aortic rami change their position relative to one another and to the vertebral column. As these organs are drained originally by paired veins, the postcardinal veins, changes in these veins associated with migration of these organs have an important bearing on the arrangement of the vessels responsible for the formation of the inferior vena cava.

## MATERIAL AND METHODS

Human embryos ranging from 6 to 35 mm. in length were used for this investigation. They are listed in Table 1.

**Table 1**

| Embryo | C-R length mm. | Embryo | C-R length mm. |
|---|---|---|---|
| I | 6 | VIII | 18 |
| II | 6 | IX | 20 |
| III | 8 | X | 20 |
| A1 | 11 | A2 | 25·6 |
| IV | 12 | L2 | 25 |
| L1 | 12 | XII | 26 |
| V | 14 | XIII | 30 |
| VI | 15·5 | XIV | 35 |
| VII | 18 | | |

I am greatly indebted to Prof. M. W. Woerdeman and Prof. J. A. J. Barge, Directors of the Departments of Anatomy in Amsterdam and Leiden Universities respectively, for the loan of the 'A' and 'L' series of embryos.

Two of the embryos were cut sagittally and the remainder transversely. They were stained with haematoxylin and eosin or by the Bodian silver impregnation method.

The embryos were studied in serial sections—still the best method for investigating the development of the veins in man (Gladstone, 1929). Wax-plate reconstructions were not made, but linear reconstructions were found to be useful in studying topographical relations.

## RESULTS

12 mm. Embryo, L1. At this stage the postcardinal vein is still the chief vein of the dorsal body wall, but it has lost part of its intersegmental drainage due, in my opinion, to two causes: the development of the suprarenal gland, and the separation or loosening of the mesonephros from the dorsal body wall, so that the postcardinal vein comes to be squeezed between the suprarenal gland and the mesonephros.

The suprarenal gland extends at this stage from the 5th to 11th thoracic segment; its topographical relations are shown in Fig. 2, in which the postcardinal vein is seen ventro-lateral to the sympathetic ganglia. In the figure another vein is seen ventro-medial to the sympathetic ganglia. It extends from the 11th to the 5th thoracic segment and there opens into the postcardinal vein. This vein, from its position between the dorsal aortic rami and the sympathetic trunk, corresponds to the later thoracic azygos vein and is regarded as the thoracic supracardinal of Huntington & McClure (1907), though this does not imply that it is continuous with the post-renal division of the inferior vena cava. A third, small, vein can be seen ventral to the suprarenal gland and medial to the Wolffian body—the subcardinal vein of Lewis (1902). The right and left subcardinal veins are joined at the level of the 12th thoracic segment by an extensive anastomosis. In later stages a migration of this anastomosis occurs.

While in the thoracic region it is the suprarenal gland that is of interest to us, in the lumbar region it is the permanent kidney that demands our attention. In the 12 mm. stage the kidney lies ventral to the bifurcation of the aorta, and its cranial pole is dorsal to the caudal end of the mesonephros (Fig. 10*a*). The postcardinal vein, dorsal to the kidney, receives small branches from the Wolffian body, one of which is seen in Fig. 4 (*c.m.v.*). It seems as if the original single postcardinal vein had been split into two, and it is interesting to speculate whether the migrating kidney is at all responsible for this. Comparison of 6 and 12 mm. embryos suggests that such may be the case. Figs. 3 and 4 show such a comparison. The kidney, at first lying below the Wolffian body, shifts its position, as indicated by the arrows in Fig. 3, so as to lie dorsal to this organ and ventro-lateral to the vertebral column. At the same time there is a caudal movement and a reduction of the Wolffian body, accompanied by a detachment of it from the dorsal body wall, thus giving it a sort of mesentery. It seems as if the dorsal part of the dorsal body wall starts moving cranially and the ventral part caudally, which, as will be seen, affects the venous system.

Fig. 4 shows the condition in a 12 mm. embryo, with one vein between the kidney and the Wolffian body and another dorsal to the kidney and in the line of the original single postcardinal vein shown in Fig. 3. This line has been called the thoracolumbar line by some authors, e.g. Reagan (1920, 1927) and Gladstone (1929).

Hochstetter (1893), the pioneer in the investigation of this venous problem, lays great stress on the influence of the kidney, though he does not give details of the process of migration. But he speaks of the cardinal vein as having a ventral and a dorsal limb, as if it had been in his mind that it was divided into two at its caudal end.

Huntington, McClure and others have regarded this dorsal limb of the postcardinal vein as a new formation, the lumbar supracardinal system, but this does not seem correct, for the dorsal limb is not new, occupies the original position, and is directly continuous with the main postcardinal vein. Gruenwald (1938) also emphasized the fact that the supracardinal system does not take part in the formation of the inferior vena cava, but according to the above his conclusion that a so-called sacrocardinal vein forms the caudal division of the inferior vena cava cannot be accepted, for this would also mean that this vein is another formation. The data obtained after an examination of pig embryos (Auër, 1942) give the same evidence.

The dorsal limb is intimately related to the kidney, curving round its dorsal side as can be seen in Fig. 11*a*, and this curve straightens out as the kidney moves cranially.

Transverse sections at the level of the cranial pole of the kidney show a small vein, appearing first at the 12 mm. stage, which runs almost vertically from the postcardinal vein to the subcardinal. Huntington & McClure (1920) call this the supracardino-subcardinal anastomosis, but it should be more properly called a postcardino-subcardinal anastomosis.

It is thus seen that in the lumbar region as in the thoracic, the original paired system of postcardinal veins, still present in the 11 mm. embryo (Fig. 5), is replaced by two veins. In the thoracic region these are the azygos and the postcardinal, and in the lumbar region the caudal mesonephric and the postcardinal.

18 mm. Embryo, VIII. At this stage the cranial part of the postcardinal that was embedded in the mesentery of the mesonephros has almost disappeared. Similarly, the caudal mesonephric branch in front of the kidney is much reduced, owing to the drainage from this part of the mesonephros being taken over by the subcardinal vein. The main caudal postcardinal has rather increased in size, and has come to lie medial rather than dorsal to the kidney, due to the movement of the latter.

The change that has occurred in the position of the kidney can be seen by comparing Figs 4 and 6, which show that at the 18 mm. stage, the kidney

has moved considerably dorsally and laterally. It lies at this stage at the level of lumbar vertebrae 1–3.

The postcardino-subcardinal anastomosis is much enlarged and now forms a wide channel, but it still lies caudal to the level of the intersubcardinal anastomosis (Figs. 10*b* and 11*b*).

Stages older than 18 mm. of which we mention the 20, 25 and 30 mm. stages, do not show any changes with regard to the level of the kidney relative to the vertebral column but as Figs. 6 and 7 demonstrate, a dorsal movement of the organs is still active.

35 mm. Embryo, XIV. There is at this stage a considerable increase in the transverse diameter of the body, whereby the kidneys come to lie at the sides rather than in front of the vertebral column (Fig. 7). The mesonephros has become more detached from the dorsal wall, thus defining more clearly its mesentery.

The postcardino-subcardinal anastomosis has become wider and shorter and runs more horizontally than at the 18 mm. stage.

Thus in the lumbar region there is a movement of the metanephros and of the mesonephros in opposite directions, and this is associated with a lengthening of the postrenal segment of the postcardinal vein (or inferior vena cava), with a change in the direction of the postcardino-subcardinal anastomosis and with a change in position of the inter-subcardinal anastomosis and with a change of the postcardinal vein relative to the kidney.

Though it appears, from a comparison of Figs. 3 and 4, that the migration of the kidney is responsible for the splitting of the post-cardinal vein into a ventral and a dorsal portion, yet the two processes are in reality independent and both are part of a general developmental process.

Evidence for this statement is furnished by an abnormal embryo of 15·5 mm. that showed total absence of the metanephros and almost complete absence of the mesonephros, the latter being recognizable only at its cranial end. Yet in this specimen development of the postcardinal and subcardinal veins and of the anastomosis between the two has proceeded in the normal way, and the anastomosis has changed its direction from a vertical to a horizontal just as in a normal embryo (Figs. 8 and 9).

*The azygos system.* In embryos of more than 12 mm. length this system is represented in the lumbar region by a longitudinal trunk lying between the sympathetic trunk and the dorsal rami of the aorta.

Gradually a plexiform system of veins appears between the two aortic dorsal rami (Strong, 1925; Reagan & Robinson, 1926). In Fig. 10 this plexus is drawn as a single vessel. It is convenient to refer to these azygos veins as lumbar supracardinals, for they lie in the same line as the thoracic supracardinals, i.e. between the sympathetic trunk and the dorsal aortic rami.

The anastomoses connecting the lumbar azygos, or supracardinal, veins are shown in Fig. 12. They anastomose with the veins of the dorsal aortic plexus and with the caudal postcardinal, or postrenal vena cava as it may now be called. This latter anastomosis, on the right side, is a very wide connexion more of the nature of a confluence than a simple anastomosis.

Reagan & Robinson (1926) have pointed out that the lumbar azygos veins disappear almost entirely as separate entities. Together with the dorsal aortic plexus (subcentral vein of Frazer) they form the terminal parts of the definitive left lumbar veins.

*Continuity of thoracic and lumbar azygos systems.* This difficult question is connected with the migration of the suprarenal gland, but though the development of the gland has been described by Wiesel (1902), Kohno (1925) and Politzer (1937), I have been unable to find any detailed account of its migration. The movement that this gland undergoes can be seen in Figs. 10 and 11. At the 12 mm. stage it extends from the 5th to the 11th thoracic vertebra; at the 18 mm. stage from the 9th thoracic to the 1st lumbar; at the 35 mm. stage from the 10th thoracic to the 1st lumbar vertebra. Associated with this caudal movement is a migration in a dorso-lateral direction (Figs. 15 and 16), and there is some caudal migration and atrophy of the mesonephros. The 20, 25, 26 and 30 mm. stages all show these changes to an extent relative to their age.

As atrophy of the cranial end of the mesonephros goes on, so there is a disappearance of the thoracic division of the postcardinal. We are thus left with a single pair of veins in the azygos line.

In the 12 mm. embryo this azygos vein extends from the 11th to the 5th thoracic vertebra, where it opens into the postcardinal; thus in the 18 mm. embryo the vein in the thoracic region is formed, as far as the 5th thoracic vertebra, by the original azygos (medial sympathetic vein of Robinson & Reagan) and, cranial to the 5th thoracic vertebra, by the thoracic postcardinal. But the thoracic postcardinal vein originally lay in the thoraco-lumbar line and not in the azygos line. It is, however, pressed in a medial direction, I believe, by the developing pleural cavities, and thus comes to lie in the azygos line (Fig. 13).

No other vein than this was found in the thoracic region, and I was unable to identify the transient vein of the thoraco-lumbar line to which Gladstone refers. I may have missed the stage at which it is most developed, but in view of my closely graded series of embryos this seems unlikely.

It is thus seen that veins of the azygos line are present in the thoracic and lumbar regions, and I have nothing to add to the account by Gladstone (1929) and by Reagan and his co-workers of the

manner in which the two chains are linked together. Though there was a direct continuation of lumbar and thoracic azygos systems through a small channel in the 18 mm. embryo, I was unable to recognize it at the 35 mm. stage.

*The circumaortic venous ring.* This was first described by Huntington & McClure (1920). Fig. 12 shows a composition of the ring that differs from the classical description of the above authors in some respects, especially with regard to the post-cardino-subcardinal anastomosis.

It has been shown that this anastomosis changes from a vertical to a horizontal direction and that this is associated with the migration of the kidney, the suprarenal gland and the mesonephros. This migration is accompanied by a shifting of the post-cardinal vein in a cranial direction and a shifting of the subcardinal in a caudal direction as is indicated by the arrows in Fig. 11*a*. The result is that the inter-subcardinal anastomosis also moves caudally. It is at the level of the upper border of the 12th thoracic vertebra at the 12 mm. stage, at the level of the 1st lumbar vertebra at the 18 mm. stage, and at a little lower down the 1st lumbar vertebra at the 35 mm. stage. This is the level of the left renal vein in the adult and thus it seems that by the 35 mm. stage migration has ceased.

Another factor involved in the migration of the kidney and affecting the topographical relations of the veins is the ventro-dorsal flattening and the associated increase in transverse diameter of the body. This ventro-dorsal compression is a symptom of the general change in diameter of the embryo as described by Brockmann. It is similar to the well-known phylogenetic change seen in the mammalian as compared with the human thorax (see Weidenreich, 1923). As a result of these changes of body form (Figs. 14–16) the kidney moves laterally; the distance between the subcardinal and postrenal division of the inferior vena cava is reduced; the subcardinal vein (prerenal division of the inferior vena cava) is brought into line with the postrenal division; the horizontal renal division (postcardino-subcardinal anastomosis), which originally has a vertical course (12 mm. stage), is thus brought into line, with the other divisions. Here we have a typical form of the development in a roundabout way, a principle in ontogeny, which has been introduced by Nauck (1931), who has already given us many striking examples of this interesting mechanism. To appreciate this change we refer to Fig. 11*a*, *b* and *c*.

## DISCUSSION

Brockmann, as well as referring to a 'sliding plane' in the movement of the abdominal viscera, mentions a 'compression' as a result of the changes in the ventro-dorsal and transverse diameters of the body. I would quite agree with this 'compression' being an agent in causing visceral movement, but cannot support his 'sliding plane' theory. Only a part of the 'inner layer' mentioned by Brockmann shifts cranially, i.e. the kidney and the persistent caudal part of the postcardinal vein; the suprarenal gland, the mesonephros, the testis and the subcardinal vein migrate caudally.

It seems that the forces, shown in Fig. 11*a*, tend to telescope the structures towards the plane of lumbar vertebrae 1 and 2, thus, perhaps, leaving room for the developing thoracic and pelvic contents.

It is difficult to assess the causes of these movements, but there does seem to be an inherent tendency for movement in the tissues of the dorsal wall. For example, absence of the kidneys, as seen in our 15·5 mm. embryo, does not mean absence of movement in the tissues of the dorsal wall in which the kidney normally lies, and absence of mesonephros and metanephros does not seem to disturb the arrangement of the veins such as postcardinal, subcardinal and circumaortic venous ring.

Considerable difficulty in understanding the development of the inferior vena cava has been caused by the various names that have been applied to the veins involved. McClure & Butler (1925), Reagan (1927) in particular, Gladstone (1929) and Gruenwald (1938) have all called attention to the confusing terminology.

It may, therefore, be of value to attempt to establish a clear terminology for the veins concerned.

The datum lines are the sympathetic trunk and the dorsal rami of the aorta. Lateral to the sympathetic trunk Reagan and others distinguish a 'thoraco-lumbar' drainage, which is not mentioned by Huntington & McClure. In the thoracic region this drainage line is only temporary, as Gladstone has pointed out, and I was unable to find it in my material. In the lumbar region this drainage line is identical with the line of the postrenal vena cava.

Huntington states that the supracardinal vein forms in the lumbar region the postrenal vena cava, and in the thoracic region it forms the azygos vein. Reagan and others have shown that the azygos line in the lumbar region is medial to the sympathetic trunk and the postrenal vena cava lateral. I suggest that the term 'supracardinal' be employed to designate the vein lying medial to the sympathetic trunk and the primordium of the azygos system. We should thus have a 'thoracic supracardinal' and a 'lumbar supracardinal' both in the same line and both medial to the sympathetic trunk.

With regard to the postcardinal vein, we have seen that this lies in the 'thoraco-lumbar line' lateral to the sympathetic trunk. From my investigations I am of opinion that the postcardinal vein divides at its caudal end into a caudal mesonephric vein and a dorsal postcardinal. Whether

this latter vein is regarded as the dorsal remains of the original postcardinal or whether we call it thoraco-lumbar does not make much difference in principle. What I wish to emphasize is that the ventral limb atrophies with the reduction of the mesonephros but the dorsal limb, the original vessel, persists as the postrenal vena cava, and thus I cannot agree with Gruenwald in his suggestion to introduce the name 'sacrocardinal' for what is not at all a new vein but only the dorsal remnant of the original postcardinal in the lumbar region.

## SUMMARY

1. An account is given of the course taken by the permanent kidney in its migration, and reference is made to the migration of the suprarenal gland and mesonephros.

2. These movements are part of an inherent tendency in the structures of the dorsal body wall, and movement of one tissue is not necessarily dependent on that of another.

3. There are forces causing a general movement from above and below towards the first two lumbar vertebrae, so giving space for the development of the contents of thorax and pelvis.

4. These movements affect the disposition of the veins.

5. It is suggested that the name 'supracardinal' be applied to veins lying in the 'azygos line' medial to the sympathetic trunk, thus avoiding ambiguity.

6. The postrenal division of the inferior vena cava is derived from the dorsal remnant of the original postcardinal vein. There is no need to introduce a new name for this vessel.

I wish to express my sincere gratitude to Prof. C. M. West for his kindness in helping me with the reduction in length of the original manuscript, necessary because of the paper situation.

## REFERENCES

Arey, L. B. (1937). *Developmental Anatomy*, 3rd ed. Philadelphia and London: Saunders.
Auër, J. (1942). *Proc. K. Akad. Wet. Amst.* **15**, 742.
Auër, J. (1944). *Ned. Tijdschr. Geneesk.* 88.
Brockmann, A. W. (1936). *Morph. Jb.* **77**, 605.
Brockmann, A. W. (1938). *Morph. Jb.* **81**, 21.
Broman, I. (1938). *Anat. Anz.* **86**, 195.
Butler, E. G. (1927). *Amer. J. Anat.* **39**, 267.
Cameron, J. (1911). *J. Anat. Physiol.* **45**, 416.
Frazer, J. E. S. (1924). *J. Anat., Lond.*, Proc. p. 99.
Gladstone, R. J. (1911). *J. Anat. Physiol.* **45**, 225.
Gladstone, R. J. (1912). *J. Anat. Physiol.* **46**, 220.
Gladstone, R. J. (1929). *J. Anat., Lond.*, **64**, 70.
Gruenwald, P. (1938). *Z. mikr.-anat. Forsch.* **43**, 275.
Gruenwald, P. (1939). *Anat. Rec.* **75**, 237.
Hochstetter, F. (1893). *Morph. Jb.* **20**, 543.
Huntington, G. S. & McClure, C. F. W. (1907). *Anat. Rec.* **1**, 29.
Huntington, G. S. & McClure, C. F. W. (1920). *Anat. Rec.* **20**, 1.
Kerschner, L. (1888). *Anat. Anz.* **3**, 808.
Kohno, S. (1925). *Z. ges. Anat.* 1. **77**, 419.
Lewis, F. T. (1902). *Amer. J. Anat.* **1**, 229.
Maxwell, E. V. (1928). *J. Anat., Lond.*, **62**, 84.
McClure, C. F. W. & Butler, E. G. (1925). *Amer. J. Anat.* **35**, 331.
Moskowicz, L. (1936). *Z. ges. Anat.* 1. **105**, 37.
Nauck, E. Th. (1931). *Morph. Jb.* **66**, 65.
Oertel, O. (1928). *Anat. Anz.* **65**, 312.
Politzer, G. (1937). *Z. ges. Anat.* 1. **106**, 40.
Reagan, F. P. (1920). *Anat. Rec.* **17**, 111.
Reagan, F. P. (1927). *Anat. Rec.* **35**, 129.
Reagan, F. P. & Robinson, A. (1926). *J. Anat., Lond.*, **61**, 482.
Reagan, F. P. & Tribe, M. (1926). *J. Anat., Lond.*, **61**, 480.
Sabin, F. L. (1915). *Contr. Embryol. Carneg. Instn*, **3**, 5.
Starkenstein, W. (1938). *Morph. Jb.* **81**, 8.
Strong, M. T. (1925). *Anat. Rec.* **31**, 320.
Weidenreich, F. (1923). *Amer. J. phys. Anthrop.* **6**, 1.
Wiesel, J. (1902). *Anat. Hefte*, **19**, 481.
Zumstein, J. (1896). *Anat. Hefte*, **6**, 571.

## KEY TO LETTERING

| | |
|---|---|
| *a.* | aorta |
| *a.a.* | azygos anastomosis |
| *a.b.* | aortic bifurcation |
| *a.c.* | auricular canal |
| *a.l.* | azygos line |
| *a.m.g.* | abnormal mesonephric glomerulus |
| *a.s.v.* | confluence of azygos and subcentral veins |
| *a.v.* | azygos vein |
| *a.v.c.* | aorta, with projected vertebral column |
| *at.* | atrium |
| *au.* | auricle |
| *b.* | bladder |
| *br.* | bronchus |
| *c.d.* | crus of diaphragm |
| *c.m.v.* | caudal mesonephric vein |
| *ca.p.m.* | caudal pole of mesonephros |
| *cr.p.m.* | cranial pole of mesonephros |
| *cr.pc.* | cranial remains of postcardinal vein |
| *cr.sc.* | cranial end of subcardinal vein |
| *d.a.p.* | dorsal aortic plexus |

| | |
|---|---|
| *d.a.p.l.* | dorsal aortic plexus line |
| *d.a.r.* | dorsal aortic ramus |
| *d.t.* | digestive tube |
| *g.g.* | genital gland |
| *h.-sc.p.* | hepato-subcardinal junction of postcava |
| *i.d.* | intervertebral disc |
| *i.m.a.* | inferior mesenteric artery |
| *i.sc.a.* | intersubcardinal anastomosis |
| *i.v.* | intersegmental vein |
| *k.* | kidney |
| *l.* | liver |
| *l.a.* | lumbar azygos |
| *l.a.v.* | line of azygos vein |
| *l.v.* | line of vertebral column |
| *l.pc.v.* | line of postcardinal vein |
| *lu.* | lung |
| *m.* | mesentery |
| *m.a.* | mesonephric artery |
| *m.d.* | Mullerian duct |
| *o.* | oesophagus |
| *o.b.* | omental bursa |
| *p.* | pancreas |
| *p.m.* | psoas muscle |
| *p.p.* | preaortic plexus |
| *p.s.* | plane of section |
| *p.v.* | portal vein |
| *pc.a.v.* | combined postcardinal and azygos veins |
| *pc.c.* | pericardial cavity |
| *pc.l.* | postcardinal line |
| *pc.l.pr.p.* | postcardinal line and postrenal division of postcava |
| *pc.-sc.a.* | postcardinal-subcardinal anastomosis |
| *pc.v.* | postcardinal vein |
| *pr.p.* | postrenal division of postcava |
| *pt.c.* | peritoneal cavity |
| *r.* | rectum |
| *r.c.a.v.* | region of compressed azygos vein |
| *r.v.* | renal vein |
| *rb.* | rib |
| *s.* | spleen |
| *s.c.* | sympathetic chain |
| *s.g.* | sympathetic ganglion |
| *s.gl.* | suprarenal gland |
| *s.v.* | sex vein |
| *s.v. (c.sc.)* | future sex vein (caudal subcardinal) |
| *s.v.c.* | superior vena cava |
| *sc.p.* | subcardinal division of postcava |
| *sc.v.* | subcardinal vein |
| *sp.g.* | spinal ganglion |
| *sp.m.* | spinal medulla |
| *sp.n.* | spinal nerve |
| *sp.v.* | spinal vein |
| *sr.v.* | suprarenal vein, cranial remnant of subcardinal |
| *t.a.v.* | thoracic azygos vein |
| *u.a.* | umbilical artery |
| *u.c.* | umbilical cord |
| *ur.* | ureter |
| *us.* | urachus |
| *v.* | vertebra |
| *v.c.* | vertebral column |
| *v.c.d.* | vein compressed by diaphragm |
| *v.s.g.* | visceral sympathetic ganglion |
| *v.s.r.* | visceral sympathetic ramus |
| *vn.* | ventricle |
| *w.b.* | Wolffian body |
| *w.d.* | Wolffian duct |

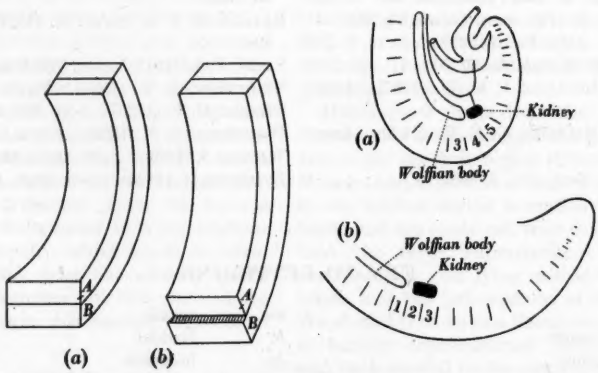



Fig. 1. Schematic illustration of Brockmann's hypothesis on the migration of the kidney. On the left, *a* and *b* represent two metal laminae fixed at the top end: in *b*, a straightening process has changed the position of the inner lamina relative to the outer. On the right, this mechanism has been transferred to the embryo. (After Brockmann.)

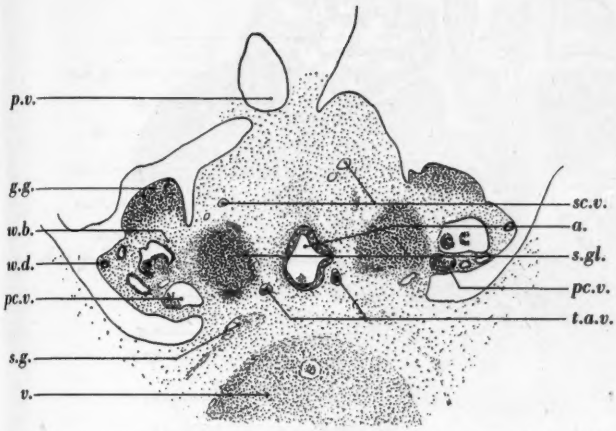



Fig. 2. Transverse section of the 12 mm. embryo showing the relations of the suprarenal gland.

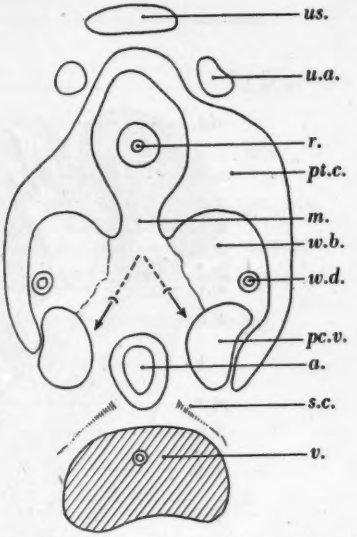



Fig. 3. Scheme of a transverse section of the 6 mm. embryo. The arrow indicates the direction in which the kidney migrates.

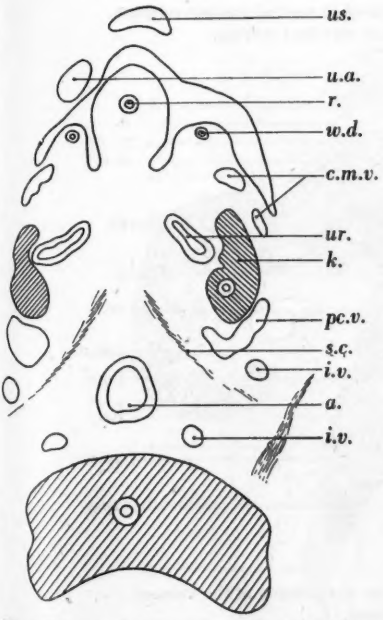



Fig. 4. Scheme of a transverse section of the 12 mm. embryo (L1). Note the position of the kidney.

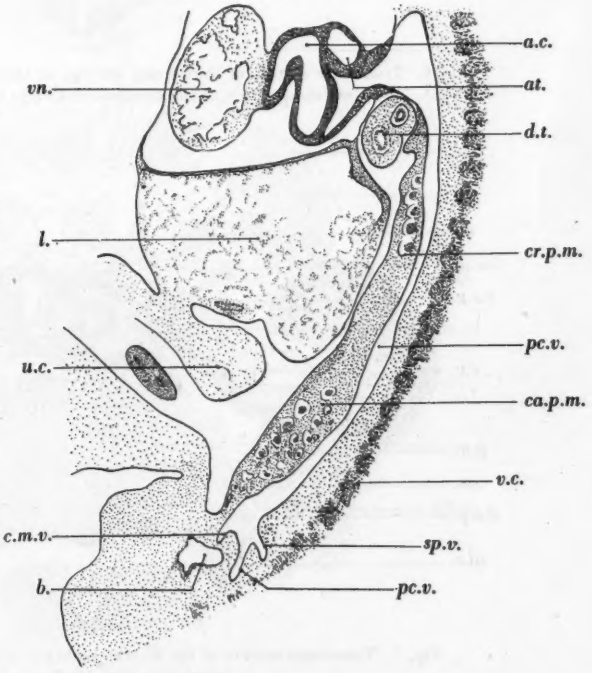



Fig. 5. Longitudinal section of the 11 mm. embryo.

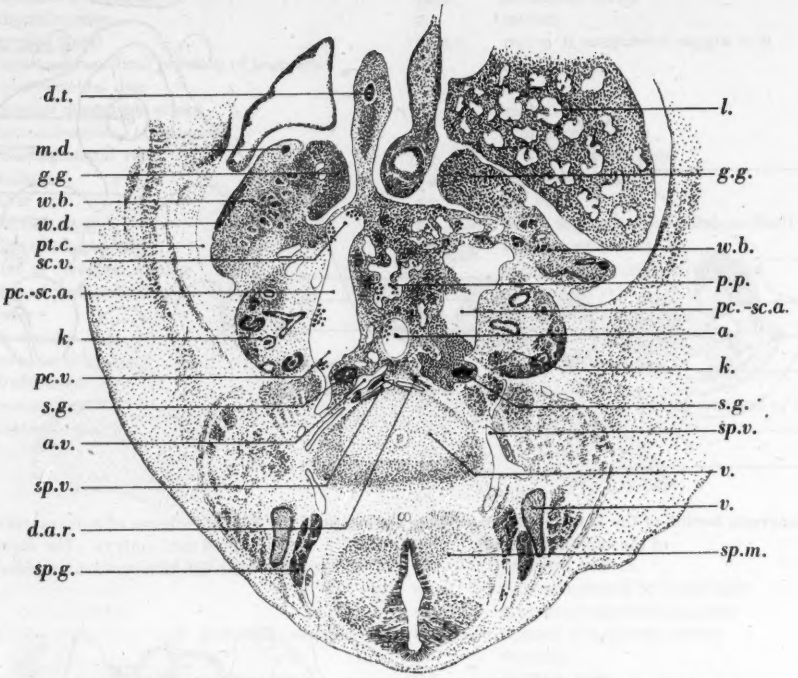



Fig. 6. Transverse section of the 18 mm. embryo at the level of the 2nd lumbar vertebra. Note the position of the kidneys relative to the vertebral column.

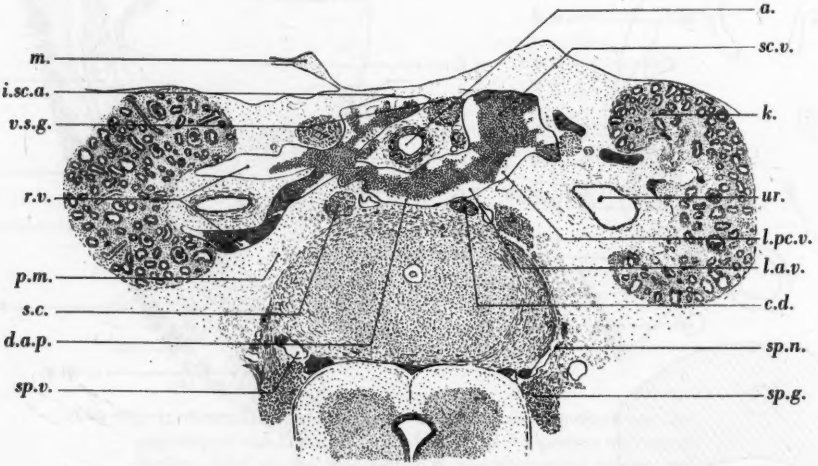



Fig. 7. Transverse section of the 35 mm. embryo. Note the position of the kidneys relative to the vertebral column.

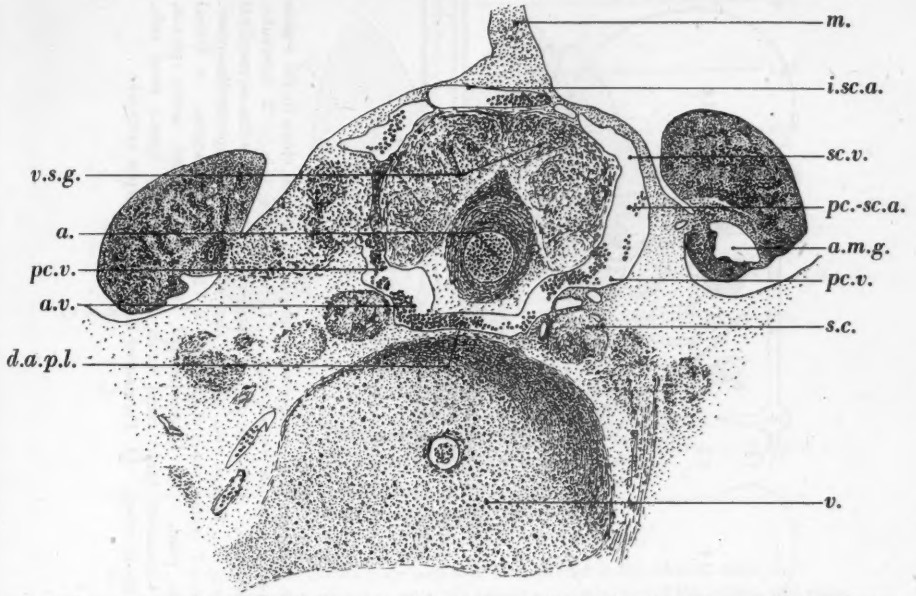



Fig. 8. Transverse section of the 15·5 mm. anomalous embryo in the region of the circumaortic venous ring Note the abnormal mesonephros and the absence of the kidney.

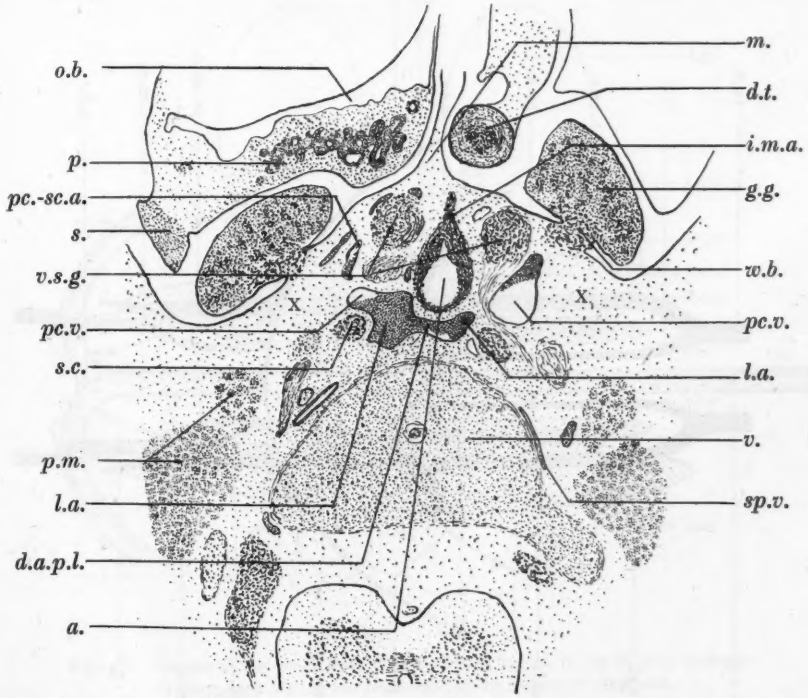



Fig. 9. Transverse section of the 15·5 mm. anomalous embryo showing normal relations of the veins below the level of the intersubcardinal anastomosis. Note the abnormal mesonephros and the absence of the metanephros (*X*).

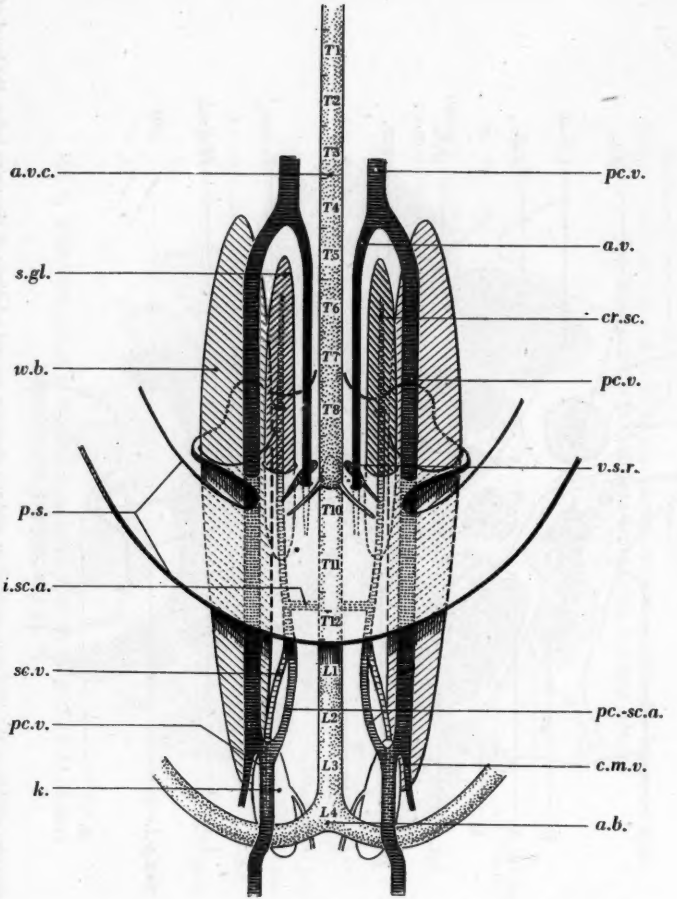



Fig. 10*a*. Scheme of the dorsal body wall seen from behind in the 12 mm. embryo (L1). A transverse section has been interposed to show the topographical relations.

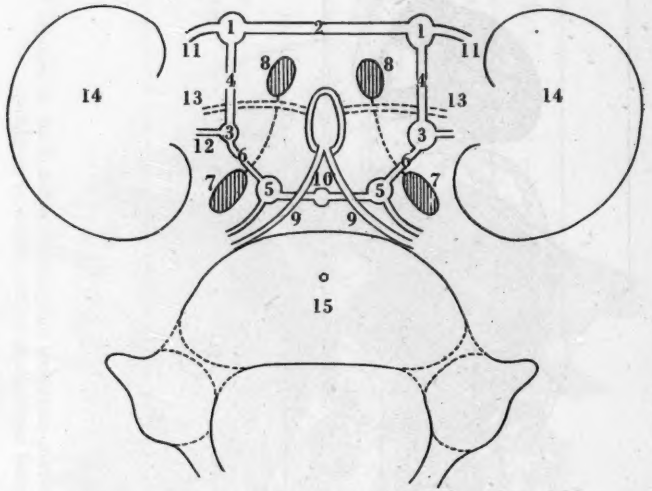



Fig. 12. Scheme of a transverse section of the 35 mm. stage in the region of the circumaortic venous ring. 1. Subcardinal vein. 2. Intersubc. anastomosis. 3. Caudal part of postc. vein. 4. Postcardino-subcardinal anast. 5. Azygos vein. (lumb. supracard. v.) 6. Supracardino-postc. anast. 7. Symp. chain of ganglia. 8. Visceral symp. ganglia. 9. Dorsal aortic rami. 10. Intersupracard. anast. with dors. aortic plexus. 11. Permanent (ant.) renal vein. 12. Temporary (post.) renal vein. 13. Renal artery. 14. Permanent kidney. 15. Corpus vertebrae.

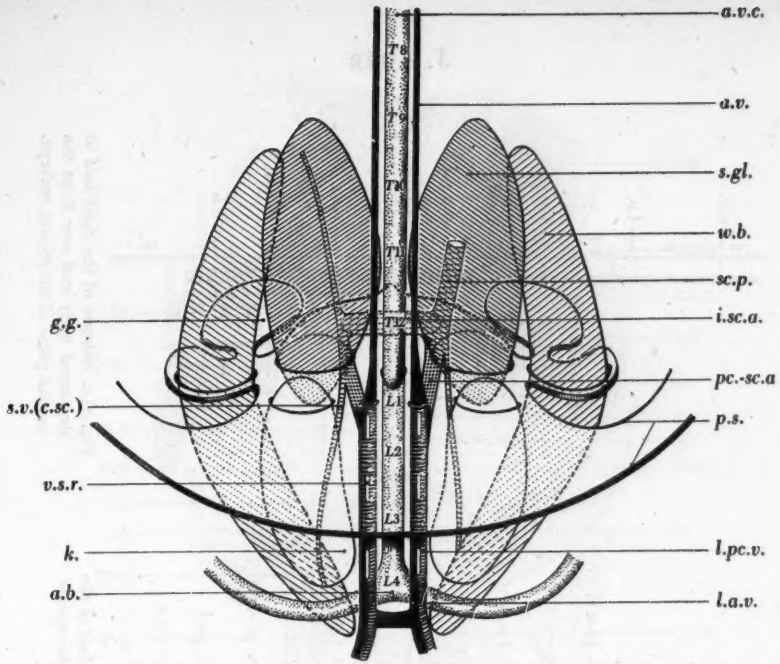



Fig. 10*b*. Scheme of the dorsal body wall seen from behind in the 18 mm. embryo. A transverse section has been interposed to show the topographic relations of the organs and veins.

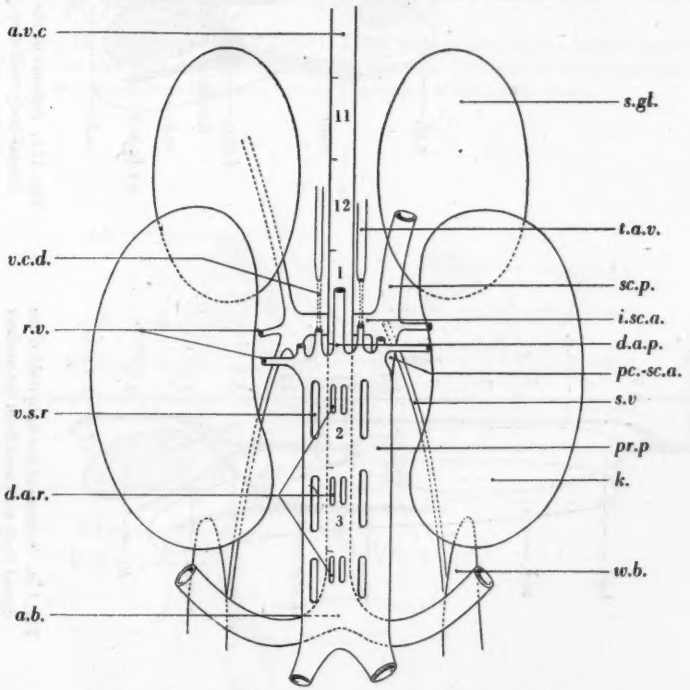



**Fig. 10***c*. Scheme of the dorsal body wall seen from behind in the 35 mm. embryo. An interposed section has been omitted for reasons of clearness.

embryo (L1). A transverse section has been interposed to show the topographical relations.

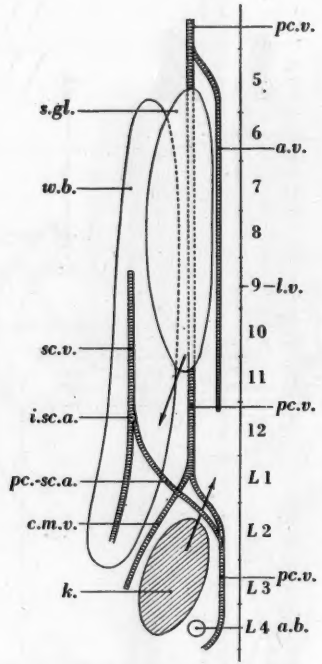



Fig. 11*a*. Scheme of the right half of the dorsal body wall seen from the median plane of the 12 mm. embryo (L1).

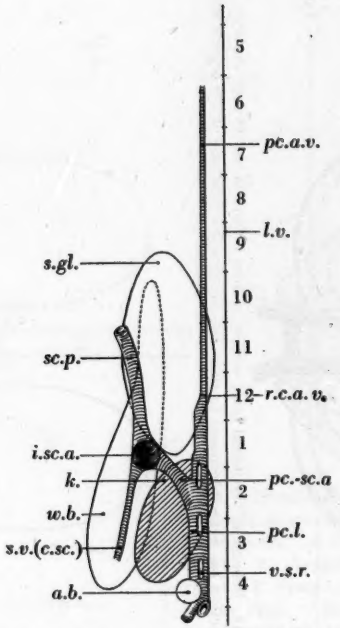



Fig. 11*b*. Scheme of the right half of the dorsal body wall seen from the median plane of the 18 mm. embryo.

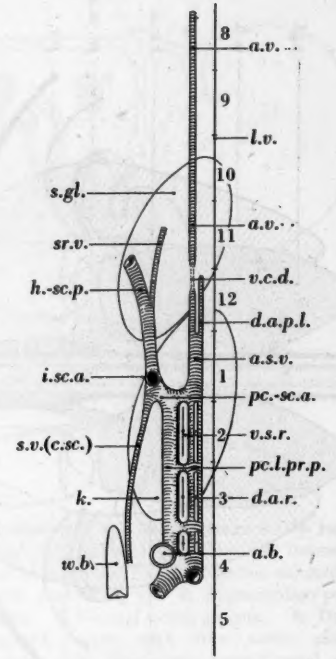



Fig. 11*c*. Scheme of the right half of the dorsal body wall seen from the median plane of the 35 mm. embryo.

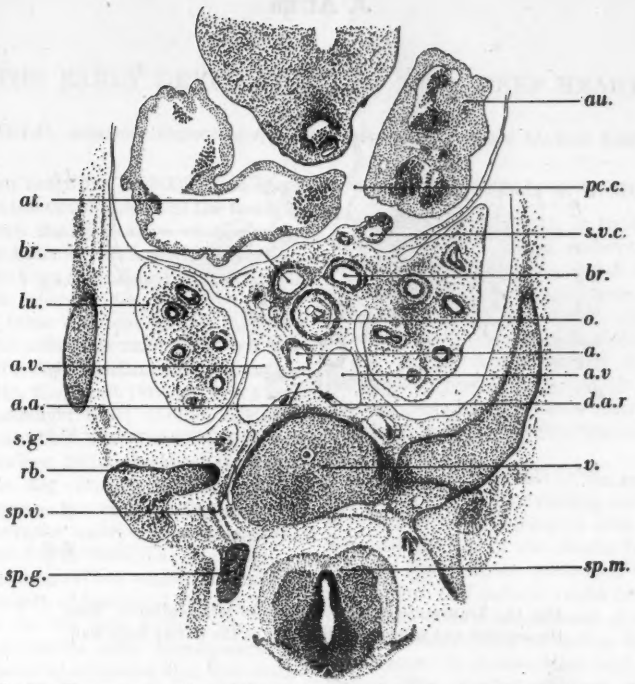



Fig. 13. Transverse section of the 15·5 mm. embryo showing the azygos venous line on a level with the third thoracic vertebra. This part of the azygos may be derived from the postc. vein (note position of the symp. chain).

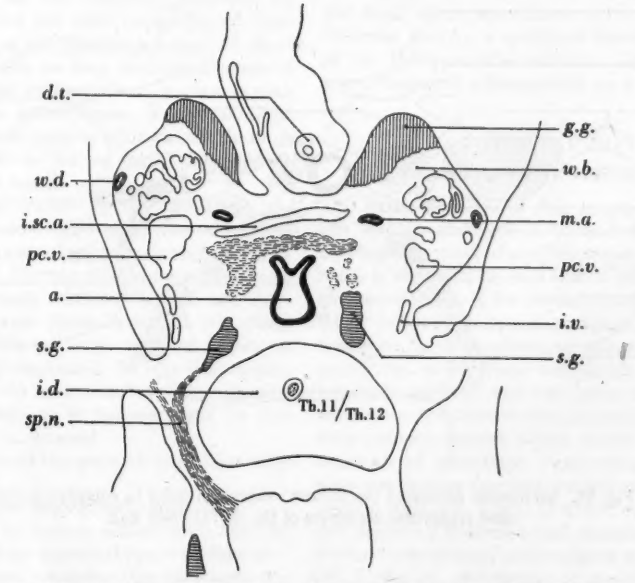



Fig. 14. Transverse section of the 12 mm. embryo (L1), for comparison of the sagittal and transverse diameters of the dorsal body wall.

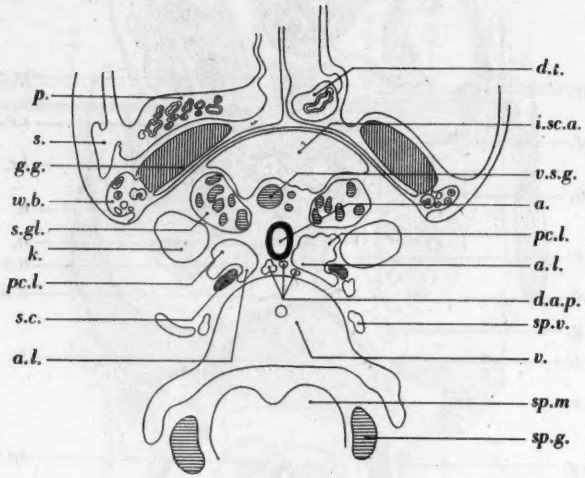



Fig. 15. Transverse section of the 18 mm. embryo. Note the sagittal and transverse diameter of the dorsal body wall.

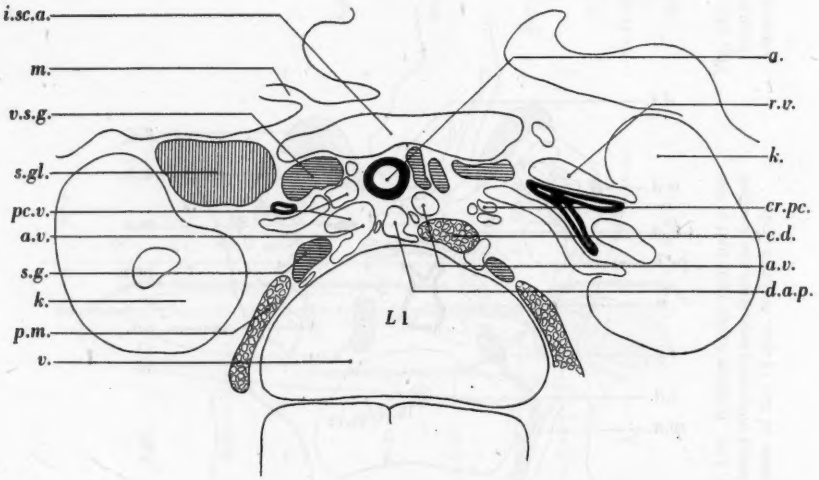



Fig. 16. Transverse section of the 35 mm. embryo in order to compare sagittal and transverse diameters of the dorsal body wall.

# THE EARLY DEVELOPMENT OF THE SHEEP HEART

By E. J. FIELD, *Anatomy Department, St Bartholomew's Hospital Medical College, London*

Since about 1880 an extensive literature has accumulated relating to the development of the heart in a variety of animals representative of the main groups; and on the basis of this knowledge certain generalizations have been established regarding the sequence of events whereby the unpaired heart rudiment arises in these different groups (Mollier, 1906). Amongst the mammals most attention has been devoted to the cat (Martin, 1902; Schulte, 1914; Watson, 1924), the ferret (Wang, 1917) and the guinea-pig (Yoshinaga, 1921), though valuable observations have also been recorded for the rabbit (Hensen, 1876; Rouvière, 1904), the sheep (Bonnet, 1884, 1889) and the dog (Bonnet, 1901) amongst others. For the human, the earliest stages are to be found in the Glaevecke embryo of von Spee and in the Sternberg and Payne embryos.

Apart from Kuhlemann (1754), whose remarkable work is nowadays largely of historical interest only, Bonnet appears to be the sole author who has recorded observations on the early development of the sheep heart in material of known age. His work is primarily concerned, however, with the evolution of the general form of the embryo, and such references as he does make to the heart are purely incidental. The writer has searched in vain for the further exposition of his findings promised by Bonnet at the end of his 1889 paper ('wird fortgesetzt'). In view of the incompleteness of these observations, especially as they indicate a mode of origin of the cardiac rudiments at variance with that established for other forms, it was thought desirable to seek confirmation of Bonnet's findings and to extend them as far as might be possible with the material at hand.

Much controversy exists in the literature regarding the relative roles played by the mesoderm and the entoderm in vasculogenesis, both inside and outside the embryo. Certain conclusions have been reached from the study outlined below, and how far these findings and those recorded by other authors are compatible with the classical theory of germ layers is briefly discussed. In this description reference is made to extra-embryonic vasculogenesis in so far only as it throws light on the endothelial problem in general.

The subject-matter of the present work thus falls under two heads:

(1) the origin of the angioblastic cells, and

(2) the sequence of events which culminate in the establishment of an unpaired heart rudiment.

The further changes whereby this rudiment attains its definitive form are not here under review.

## MATERIAL AND METHODS

The writer was fortunate in having access to serial sections of some 'timed' embryos in the possession of Profs. W. J. Hamilton and J. D. Boyd, to whom he would express his gratitude. Further, it was possible through the kind offices of Mr John Hammond, Jr., to acquire additional 'timed' embryos of the ages required for further study. Altogether three embryos of 15 days, and four of 16 days were found to show heart formation at the required stages. Earlier specimens showed no cardiac anlage.

The sheep were killed at the requisite number of days from the time of mating and the uteri excised immediately. The embryos with their membranes were washed out of the uterine horns with Locke's solution. Sometimes prolonged search was necessary before the embryo could be identified amidst the voluminous entanglement of membranes. After being photographed in saline the embryos were fixed, some in Bouin-Allen and some in Zenker-formol. The smaller embryos were embedded in agar and blocked in paraffin according to the method described by Samuel (1944), whilst the larger were treated in the ordinary way by Peterfi's double embedding method. Serial sectioning was carried out in all cases, sometimes at $7\mu$ but mostly at $5\mu$. Staining was by a modified Harris's haematoxylin or by Heidenhain's method. Eosin or orange G were employed alternatively as counterstains.

## DESCRIPTION

### *Extra-embryonic vasculogenesis*

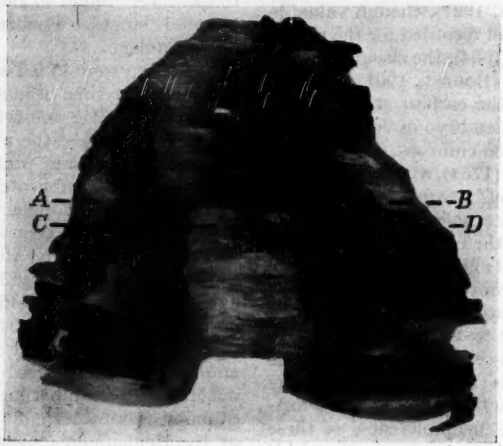
The earliest stages of this process can be seen in the 15-day embryos B 15 and B 47 L possessing four and six pairs of somites respectively. Here and there in the yolk sac wall spaces are present between the entoderm and the mesoderm (Pl. 1, fig. 1), and when followed in serial sections these spaces are found to be of spherical or spheroidal form. They are little more than well-defined and localized intervals between the two germ layers. That they are not mere artefacts is suggested by their discrete arrangement amidst tissue which shows but little evidence of shrinkage. Very soon, however, these vascular spaces become walled entirely by mesodermal cells (Pl. 1, fig. 2) which are at first plump, but assume a flattened and characteristically endothelial form as fluid accumulates within the vesicles (Pl. 1, fig. 3). Evidences of mesodermal proliferation are often to be found near such vesicles.

Entodermal participation in the wall of these primary angiocysts is thus a transient feature only, and the later angiocysts appear located entirely within the mesoderm; these vesicles may be called secondary angiocysts. The appearances are such that it seems unlikely that the entoderm makes any contribution to the definitive vessel wall; nor is blood-cell formation to be found in these early stages.

*Intra-embryonic vasculogenesis and cardiogenesis*

The general arrangement of each embryo will be described in so far as it is necessary for an appreciation of the ontogeny of the earliest vessels and of the heart.

The wax-model reconstruction (Text-fig. 1) shows that this part of the coelom has resulted from the coalescence of multiple, discrete, slit-like spaces in the mesoderm between the yolk-sac entoderm ventrally and the bulging anterior part of the brain plate dorsally (not shown in the figure). Many of these small spaces are as yet unconnected with the coelom or connected with it only by narrow and tortuous channels. The antero-posterior measurement of the uninterrupted transverse part of the coelom is about 32$\mu$. On either side it is continued into well-defined lateral channels, though here, too, there is evidence of origin from multiple spaces as indicated by the double channel seen on the left side. In effect there is in this embryo a horseshoe-




Text-fig. 1. A wax-plate reconstruction of the foregut diverticulum of embryo B 15 (15 days; four somites) together with the entoderm of the adjoining part of the yolk sac. The coelomic spaces are dark and appear as two lateral channels joined by a cranio-median limb below and in front of the foregut pouch. This cranio-median coelom is in process of formation and is represented in large part by more or less discrete mesodermal clefts. On the left side the lateral channel is double. On the right side there is a gap where slight damage to a few sections prevented the precise outline of the channel from being modelled. The small black rectangular dots indicate the position of angioblasts. The lines *AB* and *CD* are the planes of the sections shown in Pl. 1, figs. 4 and 5 respectively. The model is viewed from above and slightly behind. Magnification $\times 250$.

*B* 15. This embryo, of 15 days and possessing four pairs of somites, is the youngest in which vascular anlagen are to be found. In the anterior part of the embryonic plate changes have occurred resulting in the appearance of a shallow foregut diverticulum some 32$\mu$ in depth. The floor of this diverticulum in its posterior part projects ventrally as a gutter, just anterior to the anterior intestinal portal. It presents many mitotic figures, perhaps indicating an active 'knitting together' of the lateral coelomic folds by whose apposition, the appearances suggest, the gut has come into being. Below and in front of the anterior end of the foregut pouch is the cranio-median limb of the coelom. shaped cavity whose anterior part has just become established. Between the entoderm and mesoderm which form the floor of the distinctly patent part of the cranio-median coelom (pericardial cavity) are a few cells—some half-dozen in all—which, for reasons to be considered later, can be called angioblasts. Indeed, mitotic figures are no more numerous in the floor of the pericardium than elsewhere in this rapidly growing embryo; nor is there a heaping up of the splanchnopleure to be seen in the floor of the pericardial cavity. A section along the line *AB* in Text-fig. 1, just caudal to the pericardial cavity, is shown in Pl. 1, fig. 4. The lateral coelomic channels leading back from the lateral

extremities of the pericardial cavity are seen on either side, and four well-defined angioblastic cells can be seen 'pavementing' the entoderm. Similar cells are dusted about between the entoderm of the yolk sac and the mesoderm over the whole anterior part of the embryonic plate. Many are in relation to the coelomic clefts mentioned above, but many also are not so situated (Text-fig. 1). A section taken a little more caudally, along the line *CD* in Text-fig. 1, shows the foregut diverticulum and also some angioblastic cells in relation to it (Pl. 1, fig. 5).

Behind the anterior intestinal portal the coelomic folds stand apart, and it is in this region, where vitelline veins will come into existence at a slightly later stage, that vasculogenic cells are most in evidence. Pl. 1, fig. 6 shows such cells 'pavementing' the entoderm in a more or less continuous strand, and since similar appearances are manifest in many sections at about this level the name 'gefässblatt' employed by Bonnet is not inappropriate for the vasofactive sheet.

The chief criteria by which these cells are considered angioblastic are, first, their location close up against the entoderm—where the next embryo to be considered will show the process of their conversion into angiocysts; and, secondly, their characteristic orientation—their long axes being arranged parallel to the entoderm layer (Pl. 2, fig. 7). This alinement of the angioblasts is all the more distinctive in that the cells of the splanchnopleure forming the medial wall and floor of the lateral coelomic channel also show a definitive arrangement, their long axes being disposed radially, i.e. at right angles to the angioblasts and so producing a 'palisade' effect. No difference in nuclear or cytoplasmic staining is to be noted between the angioblasts and either the entoderm or mesoderm cells, though the angioblasts do tend to have rather scanty cytoplasm. An isolated angioblast is usually spindle-shaped.

As to the origin of these angioblasts or vasofactive cells, there is evidence of a genetic relationship with both the splanchnopleure and the entoderm. Between these two layers there is throughout a well-marked space which is no doubt attributable in large measure to shrinkage. The cells of the splanchnopleure bordering this space show signs of 'loosening', and many gradations can be seen as individual cells appear to detach themselves from the main layer and take up the orientation and position described above as characteristic of the angioblasts. It seems certain that many (probably the greater number) of the angioblasts are of such mesodermal origin. On the other hand, at many points the most intimate contact between angioblasts and entoderm exists, and an entodermal origin of some of the former seems very probable (Pl. 2, fig. 8). Certainly protoplasmic continuity between them can be established especially with the stereoscopic binocular microscope.

Traversing the 'shrinkage space' mentioned above are long or short protoplasmic fibres, some plump and others very tenuous, derived from the splanchnopleure cells adjoining the space. Some of these fibres may make contact with the entoderm cells, but most tail off and are lost in the space (Pl. 2, fig. 7). To what extent these fibres exist in the actual living condition must necessarily remain uncertain. This matter will be discussed later.

This embryo does not show a heaping up of the splanchnopleure in the floor of the lateral coelomic channel nor do the angioblasts exhibit that propensity to the formation of ring-like structures or angiocysts both of which are such prominent features in the next specimen.

*B 47 L.* This embryo, though also of 15 days, is further developed than B 15 and has six pairs of somites. The foregut has deepened to about 175$\mu$, and stretching across beneath all but the caudal 60$\mu$ of its extent is the cranio-median limb of the coelom. This, too, is 175$\mu$ in antero-posterior measurement and so projects forwards about 60$\mu$ in front of the foregut diverticulum. Evidently many of the rudimentary coelomic clefts seen in B 15 have been taken up into the pericardial cavity resulting in the considerable increase in its antero-posterior extent, and at the same time the pericardium as a whole has undergone a caudal migration either relative or absolute, which has resulted in its new position partly underlying the foregut. The guttering of the pharyngeal floor noted in B 15 is prominent especially in its more caudal part just in front of the anterior intestinal portal, and the splanchnopleure covering the ventral surface of the gut here bulges down into the pericardium. Scattered here and there in the closest relation to the steep entodermal side walls of the pharyngeal gutter are small vesicles and groups of angioblasts. One or two angiocysts occupy a median position beneath the foregut floor and represent the earliest median vascular rudiments. Such a median angiocyst is seen in Pl. 2, fig. 9, just caudal to the emergence of the coelomic channels from the lateral limits of the cranio-median coelom, and vasofactive strands are seen on both sides close up against the entoderm. The vasofactive strand consists of a chain of minute vesicles joined by solid nucleated protoplasmic bridges of angioblasts. A high-power view of a vasofactive strand is shown in Pl. 2, fig. 11. The angioblast cells are plump and as yet unflattened by accumulation of fluid. The close relation to the entoderm is well shown, and participation of this latter in vasculogenesis is almost certain. A strand of angioblasts continuous with entoderm and presumably derived from it is shown in Pl. 2, fig. 8.

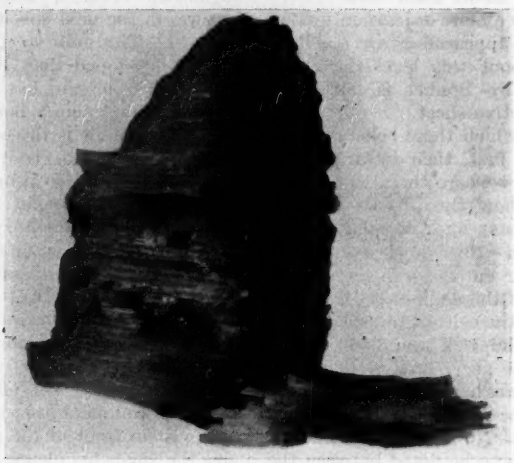
Caudal to the anterior intestinal portal where the coelomic folds stand apart, vesicle formation is much more in evidence than it is in relation to the pharyngeal floor (Pl. 2, fig. 10). This region is that in which the vitelline veins later develop and corresponds to that in which angioblasts were found to be most numerous in the preceding specimen. The vitelline anlagen are thus the most advanced in their representation at this stage.

*B* 52*B* (16 days). In this specimen, of six somites, the foregut has a depth of about 182$\mu$, and the transverse coelom stretches across beneath its anterior 105$\mu$. The pericardium extends forwards as far as the anterior end of the foregut diverticulum. It does not, as in the previous specimen, project beyond it and is 105$\mu$ in antero-posterior measurement. The pericardial cavity thus appears to have undergone a further caudal shift in relation to the pharyngeal floor. The caudal part of the foregut floors shows ventral guttering as in the preceding embryo, and the general conformation of the embryo and the coelom does not differ materially from B 47L. The vesicular vascular primordia which were noted in this latter, however, are considerably more advanced in their development. A model of the foregut and the angiocysts related to it is shown in Text-fig. 2. The whole ventral aspect of the foregut is beset with bleb-like angiocysts, many of them discrete but many, on the other hand, confluent to form what may be termed a 'subpharyngeal lake'.

In its more caudal part this median vascular rudiment is projected ventrally in the midline in adaptation to the guttering of the pharynx and is uniformly covered with splanchnopleure.

As this broad confluent collection of angiocysts is traced forwards its form is no longer seen to follow slavishly the contour of the gut floor, for, even where the latter shows but little guttering, the endocardium covered with its layer of splanchnopleure projects as a 'keel' into the pericardial cavity (Pl. 2, fig. 12).

From the front end of the subpharyngeal blood lake there pass forwards two more or less distinct vessels beneath the most anterior part of the foregut; these must be ventral aortae. They, too, are in close apposition to the entoderm. No well-defined branchial arch vessels are discernible though perhaps the angiocystic tracks indicated at *A* in Text-fig. 2 may be such rudiments.



Text-fig. 2. A wax-plate reconstruction of the foregut diverticulum of embryo B 52B seen from below and the right. The vesicular cardiac rudiment is seen besetting the pharyngeal floor. Caudally two vasoformative strands diverge to either side of the anterior intestinal portal. The strand *A* may be the precursor of a branchial arch artery. Magnification ×250.

*B* 51*L* (15 days). Although a day younger than the preceding embryo this specimen is more advanced. Unfortunately, it sustained damage in preparation. There is in it, however, a definite capillary U-loop beneath the foregut.

*B* 52*A* (16 days). This embryo has the same general conformation as has B 47L and like it has six pairs of somites. The foregut diverticulum has a depth of about 255$\mu$ and the pericardial cavity extends beneath it for 120$\mu$. Caudal to the anterior intestinal portal where the coelomic folds stand apart the vitelline veins are seen. They appear as

confluent angiocystic chains, and the calibre of the endocardial tube so formed varies considerably at different levels. This disparity can also be seen on the two sides (Pl. 3, fig. 13). Over the endothelial tube the splanchnopleure bulges into the lateral channel, and it is of interest to observe that this bulging of the medial coelomic wall may occur independently of the presence of a subjacent tube as is seen on the left side of the figure. The 'vitelline ridge', as it may be called, cannot, then, be due to the mere mechanical presence of the endothelial tube.

There are two other features relevant to cardiac development to be noticed in this specimen. The first is the presence of a distinct reticular formation or mesostroma around the endothelial tube connecting it with the overlying splanchnopleure. This meshwork is much more distinct than the faint mesostroma which was seen partially to invade the 'shrinkage space' between mesoderm and entoderm in B 15, and is apparently a further development of it. Further, it is continuous with the fibrillary stroma which is related to the median heart rudiment. The second feature is that the vitelline vein at many points stands away a little from the entoderm, as is seen on the right in Pl. 3, fig. 13, and does not bear the same close and constant relation to this layer that the cardiac endothelium does (Pl. 3, figs. 14, 15).

As the vitelline veins are traced cranialwards they assume the form of a series of contiguous and partially continuous angiocysts applied to almost the whole medial wall of the coelomic channel. Thus when communication between the vessels of each side takes place cranial to the anterior intestinal portal, a broad space is formed showing traces of its origin by the fusion of initially discrete angiocysts. In cross-section this space has the appearance of a capillary U-loop subjacent to the foregut entoderm (Pl. 3, fig. 14). This is an elaboration of the conditions obtaining in the embryo B 52 B, and indicated in the model (Text-fig. 2). This broad vascular rudiment underlying the pharynx is to be looked upon as the heart primordium. How closely the endocardium is related to the entoderm is well shown in Pl. 3, fig. 15; and here, too, the character of the fibre meshwork related to the non-entodermal aspect of the endocardium is apparent. This meshwork, together with the enclosing splanchnopleure, is the early myo-epicardial mantle. This latter follows faithfully the contour of the endocardium and shows no independent variations in surface form, though its compact outer layer does exhibit localized differences in thickness. As the endothelial heart rudiment is traced forwards it is seen to conform closely to the shape of the foregut floor. Even in its anterior part, where in B 52 B (slightly younger) the endocardium was seen to free itself from the influence of the foregut dorsal to it, the endocardium in the present specimen continues to accommodate itself in this way. Primitive vessels may be recognized passing dorsally from the forepart of this lake-like heart round the foregut wall.

A section taken 40$\mu$ in front of the anterior intestinal portal shows that as the lateral coelomic channels approach each other to break through beneath the rudimentary heart, there is left between them a septum of splanchnopleure (Pl. 3, fig. 16) which is looked upon by the writer as a ventral mesocardium; it will be further considered below.

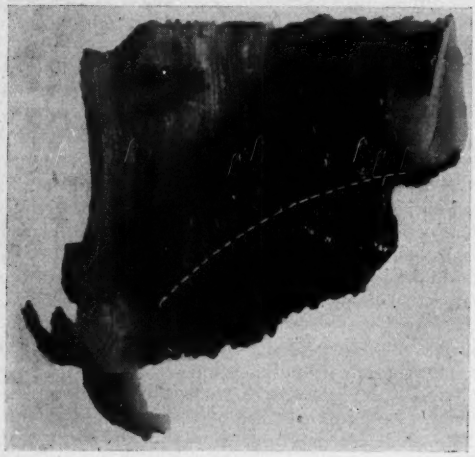
From the conditions described above it will be appreciated that there can be no question of the existence of a dorsal mesocardium at this stage, for the broad lake-like heart is applied in a sessile manner to the foregut entoderm and is in no wise 'suspended' from it.

*B* 16 *R* (16 days). In this specimen the foregut extends forwards through 368$\mu$. The pericardial cavity underlies it from a point 72$\mu$ in front of the anterior intestinal portal to a point about 96$\mu$ behind the anterior extremity, so that the cavity itself is 200$\mu$ in antero-posterior measurement.

Caudal to the anterior intestinal portal the vitelline veins are found on either side as well-formed endothelial tubes, each being surmounted by a cushion of splanchnopleure which projects into the lateral coelomic channel (Pl. 3, fig. 17). The fibre meshwork between endothelium and splanchnopleure is present though it is not so well marked as in the preceding specimen. The two venous channels are separated from the entoderm by distinct intervals occupied by the same mesostroma. Two small vessels visible more dorsally are found, by tracing them up through the series of sections, to become continuous with the dorsal extremities of the U-shaped heart. The few scattered cells between the coelomic folds in the lower part of the section indicate the imminent fusion of these folds which occurs, indeed, a few microns more anteriorly.

Anterior to the anterior intestinal portal fusion of the two vitelline veins takes place producing an endothelial channel beneath the foregut floor. This confluence of the vitelline veins is regarded as the caudal part of the heart, and the broad band of splanchnopleure below is looked upon as a ventral mesocardium. These appearances are shown in Pl. 4, fig. 18, taken 40$\mu$ in front of the portal. The myo-epicardial mantle in this and the succeeding figure is seen to exhibit changes in contour independent of those of the endocardial tube. Pl. 4, fig. 19 is of a section taken 120$\mu$ in front of that shown in the previous figure and portrays the heart U-formation at its highest development in this embryo. The close apposition of the endocardium to the foregut entoderm is well shown in Pl. 4, fig. 20, a high-power view of the same section. In it

the flattened endothelial cells are clearly seen 'pavementing' the low columnar entodermal cells. Incomplete septa derived from the contiguous walls of the original angiocysts project here and there into the lumen like baffle-plates and account for the cellular material seen scattered within the lumen. This median fusion of the two vitelline veins extends forwards for some 120$\mu$ gradually narrowing anteriorly, and throughout this extent the blood space reaches up the side wall of the gut to about the same level as in Pl. 4, fig. 19. The name 'subpharyngeal lake' is therefore well merited. From the sides of the anterior part of this space channels stretch round the gut wall and are recognized as aortic arches.



Text-fig. 3. A wax-plate reconstruction of the foregut pouch of embryo B 16R viewed from below and the right. The position of the foregut floor is indicated by the dotted line. The heart rudiment has begun to bulge ventrally, and the wide extent of its application to the entoderm is illustrated. Caudally the vitelline veins are seen skirting the anterior intestinal portal. Magnification $\times 250$, reduced to $\times 140$ for reproduction.

The myo-epicardial mantle shows localized thickenings in its wall. In addition, it shows for the first time a surface contour not entirely dependent on that of the enclosed endocardium; this is seen in Pl. 4, fig. 19 (left side), where a distinct groove is found on the upper part of the heart surface.

*B 16L* (16 days). The foregut diverticulum in this embryo of eight somites extends forwards some 280$\mu$—considerably less than in the twin embryo (B 16R) just described. The general form is similar to that of the latter, but the present embryo is definitely more advanced. The pericardium begins 80$\mu$ anterior to the anterior intestinal portal and has an antero-posterior measurement of 200$\mu$, so that it underlies the foregut floor save in its caudal 80$\mu$, and does not project beyond it distally. The heart region of this embryo has been reconstructed in wax. In it the ventral mesocardium remarked upon in the last specimen is quite distinct in relation to the caudal part of the heart. It is wedge-shaped with the base opposite the anterior intestinal portal. A section just behind the apex of the wedge, 72$\mu$ anterior to the intestinal portal, besides showing the mesocardium presents other features relevant to the heart development (Pl. 4, fig. 21). On the left surface of the myo-epicardium a groove runs forwards from the bay above the 'vitelline ridge', and this groove is the beginning of the demarcation of the heart into venous and arterial segments, or in other words indicates the beginning of a cardiac loop with convexity directed forwards, ventrally and to the right; and secondly, the 'arch' artery leaving the left part of the median heart is considerably larger than that from the right part. A branch from the left arch passes across ventral to the pharynx immediately beneath its entoderm and joins the right outflow. The origin of the right branch is, moreover, rather caudal to that of the left branch. These points are being further investigated. Thirdly, the loose cellular part of the wall of the left outflow is in marked contrast to the dense cellularity of the ventral portion of the cardiac loop.

The stages which have been described may be here recapitulated. Strands of angioblasts make their appearance at the 15th day at a time when the foregut diverticulum has just come into existence. The cranio-median limb of the coelom ('pericardial cavity') is yet incompletely formed and there is no evidence of cardiogenesis in its floor. The foregut elongates and the pericardium comes to underlie it. The strands of angioblasts meet in front of the anterior intestinal portal and in their course angiocysts develop. The vasculogenic strands become progressively more cystic, the change being more advanced in the more caudal parts—where vitelline veins will be recognized later—and in this way a median vascular rudiment closely applied to the foregut floor entoderm, and formed of contiguous and partially continuous blebs, comes into existence. An extensive subpharyngeal blood lake is formed by continued confluence of these vesicles, and in its posterior part it possesses a ventral mesocardium. No dorsal mesocardium is present in these early stages.

## DISCUSSION

### *Extra-embryonic vasculogenesis*

The description given above of early vessel formation in the yolk-sac wall of the 15- and 16-day-old sheep embryo is in full agreement with the findings of Bonnet (1884). He, too, was of the opinion that

participation of the entoderm was only a transient phenomenon. In later stages, he says, the mesoderm cells bend right round and form tubes ('durch stärkeres Einbiegen zu Röhren geschlossen') and the entoderm is thus excluded from the vessels and is seen as a well-defined and regular layer. A precisely similar sequence of events is recorded by van der Stricht (1899) in the bat; and for the pig, too, Keibel (1894) established a like process. Recently, Gladstone & Hamilton (1941) have described an analogous mechanism of vasculogenesis in the yolk-sac wall of the Shaw embryo. It appears, therefore, that the process described above is one which is common to man and a variety of mammals. Duval (1897), on the other hand, claims that in the bat those cells which are destined to form blood vessels (and blood cells) can be seen to bud off from the entoderm of the yolk-sac wall. His figures 107, 108 and 109, Planche X, are certainly very suggestive of this. A re-examination of the present sheep material in the light of these figures did not, however, reveal any comparable appearances.

*Intra-embryonic vasculogenesis*

Some of the earliest researches on this problem were carried out by His on the chick embryo, and his definitive viewpoint is embodied in what is usually called the 'Einwachsungslehre'. According to this concept vessels which are first formed in the yolk-sac wall bud off nucleated protoplasmic sprouts which grow inwards and invade the embryo itself between the entoderm and splanchnopleure. By the joining up and subsequent canalization of these 'Gefäss-sprossen' the early intra-embryonic vessels come into being. From these capillaries formed round the cephalic end of the embryonic plate the heart takes origin. Although widely accepted this doctrine evoked a certain scepticism. Thus Ranvier expressed himself: 'Mais il est clair qu'aucun embryologiste n'a pu suivre ce développement continu par bourgeonnement dans le corps de l'embryon; c'est là une simple hypothèse.' As will be seen, recent work has fully justified this criticism.

Impressed by his demonstration that the first aortic arches in amphibians developed as outgrowths from the heart endothelial sacs, Rabl propounded the theory that the endothelium of all blood vessels is in the last resort derived from the original heart endothelium. Rabl's belief that 'Endothel stammt nur von Endothel' is in accordance with the His teaching, Rabl, however, postulating an intra-embryonic vasculogenic focus. As to the precise origin of the initial vasofactive cells he is silent. This theory, too, claimed many adherents, and Sobotta, from his work on bony fishes, even went so far as to evoke a His theory in reverse—for he described the yolk-sac vessels as outgrowths from the embryonic vessels; and this he did—'ohne irgendwelche Beweise für die Richtigkeit derselben zu erbringen und ohne gegenteilige Angaben zu entkräften' (Mollier, 1906).

The third theory concerning the origin of vascular endothelium is associated with the name of Rückert. He observed the apparent *in loco* formation of vasculogenic cells in selachian embryos as a delamination from the ventral part of the mesoderm. Rückert also made the very important observation that the endothelium of the aortae in these fishes had a dual origin, being derived in part from the local mesoderm and 'in geringerer Anzahl aus der dorsalen Darmwand'. Here then is an admission of a possible duplicity of source for the vascular endothelium, and it stands in sharp contrast to the restrictive teaching of both His and Rabl. Rückert's assertion that entodermal as well as mesodermal cells may contribute to endothelium formation was based not only on the development of the aortae, but also on his findings in relation to the vessels of the mandibular arches; for here, too, entoderm of the gut was found to be implicated. Many authors gave their support to Rückert's theory of the local origin of vascular endothelium, though differences of opinion were expressed regarding the participation of the entoderm. (For a summary of the literature up to 1906 see Mollier.)

In a study in which observations are so much a matter of opinion, fresh advances can come only with the introduction of a new technique. In 1914, Miller & McWhorter published their since classical work in which it was clearly demonstrated that intra-embryonic vessel formation in the chick was an *in loco* phenomenon and took place even when all continuity with the adjacent extra-embryonic region had been severed. These studies ushered in a phase of experimental work (typified by that of Stockard (1916) on *Fundulus*) which has declared itself in favour of the local origin of endothelium. That this does occur may now be taken as an established fact.

The earliest vascular anlagen in the embryos which form the basis of this paper take the form of strands of cells or even isolated cells to which the name angioblasts or vasofactive cells has been applied. The appearances they present and their characteristic disposition between the entoderm and the splanchnopleure have been set out above (Pl. 2, figs. 7, 8). They do not differ in essential characters from those described for all other animal forms investigated. Thus, von Schulte (1914), who has gone into these features at great length in his discussion of early vasculogenesis in the cat, describes these cells as 'dorso-ventrally flattened, in groups of two or three' (p. 44). His statement that they take on a slightly paler stain (p. 43) is not borne out in the present material in which no difference from the adjacent mesoderm

can be seen; a finding in accordance with that of Yoshinaga (1921) in the guinea-pig. Sternberg (1927), on the other hand, claims that the cells of the vascular anlage possess characteristic histological features. Their nuclei are described as being smaller and more deeply stained by haematoxylin, and their rather scanty cytoplasm is said to contain very fine fibrils which cause it to appear rather darker than that of the neighbouring cells. These features are not apparent in the sheep material.

The characteristic orientation of the angioblasts von Schulte seeks to attribute to a current of fluid directed 'ventromesad' between the mesoderm and the entoderm, and presumably the cells are supposed to present themselves 'end on' to the stream. To most workers, however, such a mechanism will, no doubt, appear improbable, especially when the syncytial nature of the tissue concerned is recalled. In the embryos of the present series and in those pictured by Bonnet and by von Schulte himself, as well as those shown by other authors, there exists a considerable space or series of spaces between the ventral aspect of the mesoderm and the entoderm. This space is not quite so large in Yoshinaga's figures (1921), though he, too, refers to it in his text. It is evidence of considerable shrinkage having occurred during the preparation of the material. Obviously disruptive forces of considerable violence have been at work, and any 'Entwicklungsmechanik' based upon the detailed and precise appearances of individual cells under such circumstances must necessarily be treated with caution. Probably the region between the splanchnopleure and the entoderm is indeed one rich in fluid, and this fluid may be contained within a protoplasmic meshwork which is continuous with and acts as a bridge between the two layers. The changes induced by fixation (cf. Tellyesniczky, 1898–1905), as well as those incidental to paraffin embedding, may well account for the 'interdermal cytodesmata' of Studnička (1911) or the 'fibres' of von Szily (1903) so accurately and painstakingly described. The forcible tearing apart of the germ layers might likewise be responsible for the appearances described as 'loosening' of the deepest part of the splanchnopleure, and 'detachment' of splanchnopleural cells to form angioblasts. Probably also the 'palisade' arrangement of the mesoderm cells bounding the lateral coelomic channel is in part due to the operation of these same forces which would tend to orient the cells in the manner observed. On the other hand, the angioblasts are in the closest possible relation to the entoderm (Pl. 2, fig. 8). In many places, indeed, direct protoplasmic continuity between entoderm and syncytial strands of angioblasts can be made out. Perhaps the truth of the matter may be that we are dealing here with a syncytium comprising mesoderm and entoderm as a single entity. It is interesting to observe in this connexion that Martin's (1902) figure does not show cell outlines in many places. If this interpretation be correct, then angioblastic cells arise from both the mesodermal and entodermal components of the syncytium, and the antithesis between views so hotly maintained in the past is, in large part, resolved. There can be no doubt that our ideas of the individuality of germ layers born in the heyday of early mechanistic embryology have been much modified at the hands of modern experimentalists and tissue culture workers.

The plasticity retained by cells, even in the full-grown organism, has been much underestimated (cf. Clarke (1914, 1916), and more especially Levander (1945)). Certainly the 'determination' of germ layers in the early embryo is much less rigid than has been supposed. The genealogy of a particular cell in the embryonic mesenchyme complex is of less importance in determining its fate than are its *position* and its *relations*. In the particular instance of angiocyst formation it is probable that a 'focus' appears which induces in the surrounding cells the tendency to enclose it. The characteristic orientation of the angioblasts and the disposition of the angiocysts with their long axes for the most part parallel to the entoderm suggests that powerful directing forces are at work. Some of these cells may later escape beyond the influence of the 'directing focus' and may then revert to their non-specific form. Such retrogression has long been known to occur. Huntington (1911) described such changes in connexion with the development of the perivenous plexuses—'their endothelial lining appears to revert to the indifferent type of the embryonic mesodermal cell' (cf. von Schulte, 1914, pp. 10 ff.).

### *Origin of the heart*

Our knowledge of the development of the heart in a number of vertebrate groups has been summarized, up to 1906, by Mollier in O. Hertwig's *Handbuch der Vergleichenden und Experimentellen Entwicklungslehre der Wirbeltiere*, vol. 1. In order to assess the position of the sheep in this respect it is necessary to outline in brief the essential findings in reptiles, birds and other mammals including man.

In the reptilian embryo (*Lacerta*) paired cardiac anlagen unite whilst they are yet in the form of strands of angioblastic cells, amongst which, however, fine spaces are beginning to appear. At this stage of commencing union the pleuro-pericardial channels are quite separate. Only when union has been established is a 'loosening' of the cells of the median rudiment to be observed, and this leads to a 'mesenchymatous' or 'meshwork' condition. The further development of this type of heart is complicated by a characteristic lateral bending of the embryo and does not concern us here.

In the chick embryo the cardiac anlagen take the form of angioblastic strands which run together cranial to the anterior intestinal portal, at a time when the pleuro-peritoneal channels are quite independent. In the process of union the heart strands take on a meshwork or 'mesenchymatous' appearance, but no definitive endothelial tubes come into existence before fusion is complete. The coelomic channels then extend ventrally and medially towards each other beneath the median cardiac rudiment, and in this way the ventral mesocardium comes into being as the septum between them. This mesocardium is only transient and gives way very soon. A myo-epicardial mantle of splanchnopleure clothes the ventral and lateral aspects of the fused anlagen, whilst the dorsal aspect is in broad contact with the entoderm of the foregut floor. It is important to note that in the chick the fusion of the cardiac anlagen occurs at a time when each is in the form of a meshwork of cells showing here and there a tendency to form a short segment of endothelial tube. Fusion at such a stage has been described only for the chick.

For the mammalian embryo it has been generally accepted, since the pioneer work of Hensen (1876), that the cardiac primordia are bilateral and that fusion occurs after they have taken on the form of distinct endothelial tubes with well-defined non-loculated lumina. The mechanism of approximation of the tubes is not the same in various animals, and their spatial relationships are dependent upon two factors: (1) the form of the foregut at its inception, and (2) the position occupied by the endothelial heart tubes in the floor of the lateral coelomic channels. The operation of these factors is seen in an examination of the conditions obtaining in the rabbit on the one hand and in the guinea-pig on the other. In the rabbit, the foregut when newly formed is very broad. Further, the endothelial heart tubes appear high up in the medial wall of the lateral coelomic channel. When closure of the foregut takes place the endothelial heart tubes are thus situated far apart, each being related to the lateral part of the gut floor. The fusion of the coelomic folds leads to a septal formation below the foregut. This cannot, however, be regarded as a ventral mesocardium, since the endothelial tubes are placed well on either side of the midline and preserve their independence in respect of both endocardial and myo-epicardial layers. Secondarily this septum 'gives way' and the coelomic channels communicate across the midline beneath the foregut to constitute a pericardial cavity. Into this pericardial cavity the endothelial heart tubes project from the lateral part of the pharyngeal floor. There is no indication of a dorsal mesocardium at this stage. Only secondarily do the cardiac tubes approach each other, fuse, and become suspended by a dorsal mesocardium.

The guinea-pig (Strahl & Carius, 1889), on the other hand, manifests certain differences owing to the fact that the endocardial tubes make their appearance in the floor of the lateral coelomic channel rather than in its medial wall. As a result, when these walls come together a septum is formed uniting the floor of the foregut to the 'middle cardiac plate' which intervenes between the floors of the coelomic channels of the two sides. Narrowing of the middle cardiac plate occurs as the endocardial tubes approach one another and come into apposition. Secondarily, the coelomic spaces break through from side to side beneath the pairing tubes. From this account it appears that it is not quite accurate to say that the enclosure of the foregut is the cause of the union of the heart anlagen, for this closure may yet leave a considerable interval between the two tubes.

Yoshinaga (1921) has reinvestigated the process of cardiogenesis in the guinea-pig and draws attention to the resemblances between the cardiac development seen in an embryo with three pairs of somites and that found in the Glaevecke embryo of von Spee (1889). In the former, however, the angioblasts are already beginning to group themselves into endothelial tubes at certain points, and these groupings are producing localized bulgings of the covering splanchnopleure into the lateral coelomic channels. Although endothelial tubes are by no means fully developed in this embryo, and certainly much less so than in most mammals at this stage of short foregut diverticulum, yet they are much in advance of the conditions found in the Glaevecke specimen. Vasculogenesis in this latter is, indeed, so early that to von Spee himself it seemed virtually absent: 'Gefässendothelröhren fanden sich nirgends in der Keimscheibe. Auch an der Stelle, wo man die primitiven Herzanlagen erwarten würde, fanden sich ganze vereinzelte Zellen, die aus dem festeren Continuitätsverbande der Keimblätter (vielleicht in Folge einer Verletzung) ausgetreten zwischen Mesoderm und Entodermlagen.' H. M. Evans (1912), however, interpreted these scattered cells as cardiac anlagen. The condition observed in the embryo 'Glae' are much more like those seen in the sheep specimen B 15 described above. As in the embryo 'Glae', B 15 nowhere shows endothelial tube (or even vesicle) formation, vessel rudiments being represented merely by scattered angioblasts. Pl. 1, fig. 5 is quite comparable with Evans's fig. 404, in Keibel and Mall. At a rather more advanced stage, also, the sheep resembles the human more closely than does the guinea-pig. For in a guinea-pig embryo with four pairs of somites (Yoshinaga, p. 270) union of the paired cardiac rudiments in front of the anterior intestinal portal is by angioblasts 'underneath the flat splanchnopleural folds of the cranio-median limb of the pericardial cavity', whilst 'the endothelial tubes are differentiated at great length,

extending throughout nearly the whole extent of the lateral pericardial cavity'. Although 'these tubes are irregularly interrupted, their continuity (is) bridged by angioblast cords'. Yoshinaga pictures (fig. 13) these endothelial tubes in a considerably more advanced stage of continuity than his text indicates, and certainly more so than is to be found in the corresponding sheep embryo B 47L in which the first union is occurring. Here fusion is occurring between vesicular strands (Pl. 2, fig. 9), and not simple angioblast chains as in the guinea-pig. However, the resemblances between the guinea-pig and sheep cardiac primordia at the time of fusion are notable, especially when comparison is made with the processes described in other mammals such as the rabbit, cat, dog and ferret, for in the latter group well-defined endothelial tubes are formed, and it is between these that fusion takes place.

Perhaps the most striking comparisons, however, are those between the sheep and human hearts round about the time of fusion of the paired primordia. Cardiogenesis in the Sternberg (1927) embryo (four pairs of somites) appears to be comparable in many respects with that in the sheep specimens B 47L and B 52A. In the Sternberg embryo (p. 161) the heart is represented by 'einem Netz von regellos angeordneten Strängen, in welchen zahlreiche Hohlräume ausgebildet sind... und liegt an der Ventralseite...des...Vorderdarmes, zwischen diesem und der...Perikardialhöhle'. From the lateral parts of this cardiac network vessel anlagen pass backwards on either side of the anterior intestinal portal—vitelline veins—and Sternberg suggests that these will be progressively taken up in the formation of the endocardial tube as the intestinal portal moves caudally. From the cranial end of the subpharyngeal network the anlage of the first aortic arches can be traced forwards in much the same way apparently as in the model (Text-fig. 2). The actual character of the 'Herzplexus' is very similar to that of the sheep specimen, being made up of contiguous and partially continuous angiocysts closely applied to the foregut entoderm (cf. Abb. 16, p. 163).

Davis (1927), in his review of the early stages of development of the human heart, describes conditions in embryos of two and four somites which show close resemblances to those in the sheep. However, there are one or two differences to be noted. Thus Davis describes (p. 257) the endocardium as beginning 'caudally...at the extremity of the atrium, as a blind endothelial channel, which abuts against, but apparently is not connected with, the primordial umbilico-vitelline channel extending caudally therefrom'. No such remarkable discontinuity has been found in the sheep material, and would in any case be very difficult to establish with certainty. Further, at this early stage when the heart plexus is yet 'a flattened sheet of endothelial strands' with 'vesicles, or angiocysts, more or less fused with one another', there are already demarcations in the myo-epicardial mantle giving some indication of the future atrial, ventricular and bulbar regions. In the sheep these early divisions are not to be found. In the Ingalls two-somite embryo the conditions appear to be very similar to those obtaining in the sheep specimen (Ingalls, 1920, Pl. 3 and present Text-fig. 2). However, as Davis says (p. 261), the Ingalls embryo shows a 'general tubular endocardium' at the two extremities of the heart whilst the intervening part retains the 'primitive plexiform arrangement'. This state of affairs cannot be substantiated for the sheep.

It appears then that in the guinea-pig, man and sheep, fusion of the bilateral cardiac anlagen occurs at a time when their condition has not progressed beyond the stage of vesiculated strands. The causal factor may be the early closure of the foregut with consequent approximation of the rudiments; and it is possible that this early closure may itself be referable to the comparatively small size of the yolk sac (Bonnet, 1884).

The presence of a ventral mesocardium (Pl. 4, fig. 21) in relation to the caudal part of the heart at once raises the question of the mechanism of closure of the foregut; for it was on the alleged absence of such a structure that Robinson (1902) in large part based his denial of the fusion of the lateral coelomic folds.

Bonnet-Peter (1929) shows a section through a sheep embryo with eight pairs of somites resembling precisely the above specimen B 16L, and he, too, indicates a mesocardium ventrale (fig. 319). In his text he suggests that it is formed by the apposition of the lateral coelomic folds. Without longitudinally sectioned specimens it is not possible to arrive at a definite opinion on this question, but it may be said that the appearances found in the embryos described above are strongly indicative of such fusion. So far as the human embryo is concerned the existence of a ventral mesocardium is strongly denied by Davis (1927). Opinions to the contrary are brushed aside by both Davis and Wang (1917), and the latter offers a reinterpretation of Wilson's (1914) finding of a small ventral mesocardium in a human embryo of probably three somites. Wang further records appearances identical with those shown by Wilson in Johnstone's second embryo. However, he prefers not to apply the name 'ventral mesocardium' to the band of tissue binding down the heart to the septum transversum, but merely to call it part of the septum transversum because the vitelline veins pass through it. Wang's fig. 35, nevertheless, shows a distinct endothelial tube surrounded by a muscular heart tube, and it is difficult to see why the band of tissue in question

should not be looked upon as a ventral mesocardium. In the sheep material described above the band of splanchnopleure shown in Pl. 4, fig. 21 connects a well-defined unpaired heart rudiment with the developing septum transversum and appears to be a true mesocardium.

The validity of such minute distinctions as Wang seems bent upon making is further called into question by the difficulty of demarcating precisely the limits between vitelline vein behind and 'heart' in front. Thus Watson (1924) points out that Wang uses the term 'vitelline vein' for even the anterior portion of the structures commonly called lateral heart tubes '...and also applies the term to structures which I described as lateral heart tubes in *Dasyurus*'.

The existence of a ventral mesocardium would seem to be established for the sheep but still doubtful in the case of other mammals including man. However, the general resemblances in cardiac development in the sheep and in man are so striking that it would be surprising if further study did not establish at least a small and transient ventral mesocardium in the latter.

## SUMMARY

1. An account is given of the mechanism of extra-embryonic vasculogenesis in the sheep.

2. The process of cardiogenesis is traced from its first stages up to the formation of an unpaired heart rudiment, and is found to show differences from that described for many mammals (such as the rabbit, cat and dog) but striking similarity to that which takes place in the guinea-pig and more especially in man.

3. The origin of vasoformative cells is discussed with reference to their 'germ-layer' origin.

4. The existence of a ventral mesocardium is established, and its significance indicated.

It is with pleasure that the author records his gratitude to Profs. W. J. Hamilton and J. D. Boyd for access to the sheep material made available to them by the help of a research grant from the University of London. To Prof. Hamilton he would also express his indebtedness for constant encouragement and helpful criticism, to Mr A. E. Westwood for his skill and patience in preparing the photographs, and to Mr E. J. Park for his share in the preparation of the sections. The expenses of microphotography have been met by a grant from the Thomas Smythe Hughes Medical Research Fund of the University of London, for which the author expresses his thanks.

## REFERENCES

Bonnet, R. (1884). *Arch. Anat. Physiol.* (Anat. Abt.), p. 170.

Bonnet, R. (1889). *Arch. Anat. Physiol.* (Anat. Abt.), p. 1.

Bonnet, R. (1901). *Anat. Hefte*, **16**, 233.

Bonnet-Peter (1929). *Lehrbuch der Entwicklungsgeschichte*, 5th ed. Berlin.

Clarke, W. C. (1914). *Anat. Rec.* **8**, 95.

Clarke, W. C. (1916). *Anat. Rec.* **10**, 301.

Davis, C. L. (1927). *Contr. Embryol. Carneg. Instn*, **19**, 245.

Duval, M. (1897). *J. Anat., Paris*, **33**, 1.

Evans, H. M. (1912). *Manual of Human Embryology* (Keibel and Mall), **2**, 498. Philadelphia and London.

Gladstone, R. J. & Hamilton, W. J. (1941). *J. Anat., Lond.*, **76**, 9.

Hensen, V. (1876). *Arch. Anat. Physiol.* (Anat. Abt.), p. 1.

Huntington, G. S. (1911). *Mem. Wistar Inst.*, no. 1.

Ingalls, N. W. (1920). *Contr. Embryol. Carneg. Instn*, **11**, 61.

Keibel, F. (1894). *Morph. Arb.* **3**, 1.

Kuhlemann, J. C. (1754). *Observationes quaedam circa negotium generationis in ovibus factae*, 2nd ed. Lipsiae.

Levander, G. (1945). *Nature, Lond.*, **155**, 148.

Martin, P. (1902). *Lehrbuch d. Anatomie d. Haustiere*, **1**. Stuttgart.

Miller, A. M. & McWhorter, J. E. (1914). *Anat. Rec.* **8**, 203.

Mollier, S. (1906). *Handb. d. vergl. u. exp. Entwick. Wirbeltiere* (O. Hertwig), **1**, 1020 and 1261.

Robinson, A. (1902). *J. Anat. Physiol.* **37**, 1.

Rouvière, H. (1904). *J. Anat., Paris*, **40**, 610.

Samuel, D. M. (1944). *J. Anat., Lond.*, **78**, 173.

Schulte, H. von W. (1914). *Mem. Wistar Inst.*, no. 3.

Spee, F. von (1889). *Arch. Anat. Physiol.* (Anat. Abt.), p. 159.

Sternberg, H. (1927). *Z. ges. Anat.* 1. *Z. Anat. EntwGesch.* **82**, 142.

Stockard, C. R. (1916). *Amer. J. Anat.* **18**, 227.

Strahl, H. & Carius, F. (1889). *Arch. Anat. Physiol.* (Anat. Abt.), p. 231.

Stricht, O. van d. (1899). *Bull. Acad. Méd. Belg.* **13**, 336.

Studnička, F. K. (1911). *Anat. Anz.* **40**, 33.

Szily, A. von (1903). *Anat. Anz.* **24**, 417.

Tellyesniczky, K. (1898). *Arch. mikr. Anat.* **52**, 202.

Tellyesniczky, K. (1902). *Arch. mikr. Anat.* **60**, 681.

Tellyesniczky, K. (1905). *Arch. mikr. Anat.* **66**, 367.

Wang, C. C. (1917). *J. Anat., Lond.*, **52**, 107.

Watson, K. M. (1924). *J. Anat., Lond.*, **58**, 105.

Wilson, J. T. (1914). *J. Anat. Physiol.* **48**, 315.

Yoshinaga, T. (1921). *Anat. Rec.* **21**, 239.

## EXPLANATION OF PLATES

### PLATE 1

Fig. 1. A photomicrograph of a well-defined vesicular space or primary angiocyst situated between the mesoderm above and the entoderm below. Embryo B 15, 15 days old. Magnification ×1300, reduced to ×980 for reproduction.

Fig. 2. A photomicrograph of a well-defined secondary angiocyst, the wall of which is formed entirely from mesoderm. To the left of the vesicle a mesoderm cell is in mitosis. The cells of the vesicle wall are plump and have not yet become flattened by the accumulation of fluid within the angiocyst. The entoderm is seen as the lower layer and is excluded from the angiocyst wall. Embryo B 15, 15 days old. Magnification ×1300, reduced to ×980 for reproduction.

Fig. 3. A photomicrograph of an angiocyst at a later stage of development. The increase in fluid within the vesicle has resulted in the cells of the vesicle wall assuming a typical flattened endothelial form. The entodermal layer of cells is seen below. Embryo B 47 L, 15 days old. Magnification ×1300, reduced to ×980 for reproduction.

Fig. 4. A section taken along the line *AB* in Text-fig. 1 (embryo B 15, 15 days old). The lateral coelomic channels are seen on either side just caudal to their emergence from the lateral parts of the cranio-median limb of the coelom. Four angioblasts are seen in relation to the entoderm. There is here no evidence of a 'cardiogenic plate'. Magnification ×200, reduced to ×150 for reproduction.

Fig. 5. A section taken along the line *CD* in Text-fig. 1. The foregut diverticulum is seen in the midline apparently formed by the fusion of the lateral coelomic folds. Several mitotic figures are to be found here. In relation to the lower part of the foregut floor there are a few scattered angioblasts on either side, and further laterally some are also found below the lateral coelomic channels. Embryo B 15, 15 days old. Magnification ×200, reduced to ×150 for reproduction.

Fig. 6. A section through embryo B 15 (15 days old) taken caudal to the anterior intestinal portal. On either side a string of angioblasts is to be seen 'pavementing' the entoderm. Between the latter and the splanchnopleure of the lateral coelomic channels there is a wide shrinkage space. Magnification ×200, reduced to ×150 for reproduction

### PLATE 2

Fig. 7. A section through embryo B 15 to show the intimate relationship of angioblasts to entoderm. A strand of angioblasts is seen 'pavementing' the entoderm in the lower part of the figure. The orientation of the angioblasts parallel to the entoderm is shown, as is also the characteristically 'radial' disposition of the splanchnopleure cells forming the floor of the lateral coelomic channel. The shrinkage space between entoderm and splanchnopleure is partially invaded by protoplasmic processes from the cells of the latter. Magnification ×520, reduced to ×390 for reproduction.

Fig. 8. A photomicrograph illustrating the intimate relationship between the entoderm and a strand of angioblasts. Protoplasmic continuity seems very probable. Embryo B 47 L, 15 days old. Magnification ×1300, reduced to ×980 for reproduction.

Fig. 9. A section through embryo B 47 L (15 days) just anterior to the anterior intestinal portal. Angiocystic strands are seen in relation to the entoderm of the pharyngeal floor, and in the midline below there is a well-defined vesicle. Such are the first median cardiac rudiments. Magnification ×200, reduced to ×150 for reproduction.

Fig. 10. A section through embryo B 47 L a short distance caudal to the anterior intestinal portal. Angiocystic strands are seen on either side adjacent to the entoderm (cf. Pl. 1, fig. 6). Magnification ×200, reduced to ×150 for reproduction.

Fig. 11. Part of the angiocystic strands seen in fig. 10 at a higher magnification. The character of the strand is shown. The vesicles are joined by angioblasts, and the whole chain is closely related to the entoderm. Magnification ×520, reduced to ×390 for reproduction.

Fig. 12. A section through the anterior part of embryo B 52 B (16 days). Below the foregut diverticulum is a 'keel-like' heart projecting down into the underlying pericardial cavity. Magnification ×200, reduced to ×150 for reproduction.

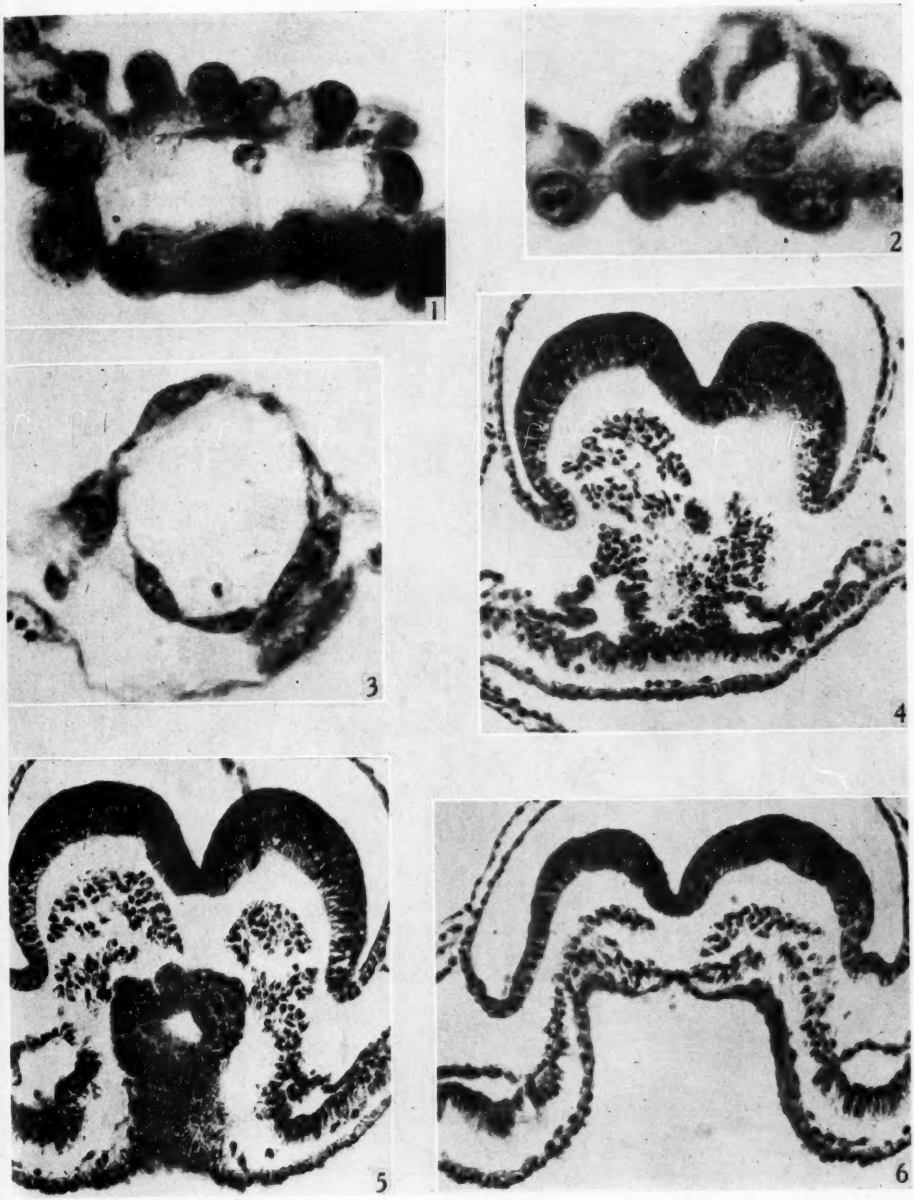
### PLATE 3

Fig. 13. A section through embryo B 52 A (16 days) just caudal to the anterior intestinal portal. The vitelline vein formations are seen on either side, the overlying splanchnopleure being heaped up into the lateral coelomic channel in each case. The mesostroma between the endothelial channels and the splanchnopleure is well defined. Magnification ×200, reduced to ×150 for reproduction.

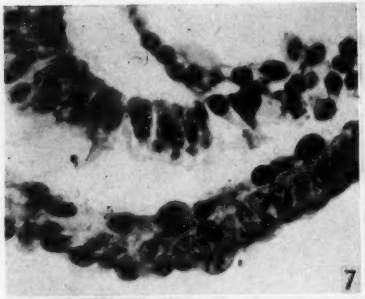
Fig. 14. A section through embryo B 52 A (16 days) in front of the anterior intestinal portal. The vesicular 'subpharyngeal lake' is seen in relation to the pharyngeal entoderm. The mesostroma of the myo-epicardial mantle is prominent. Magnification ×200, reduced to ×150 for reproduction.

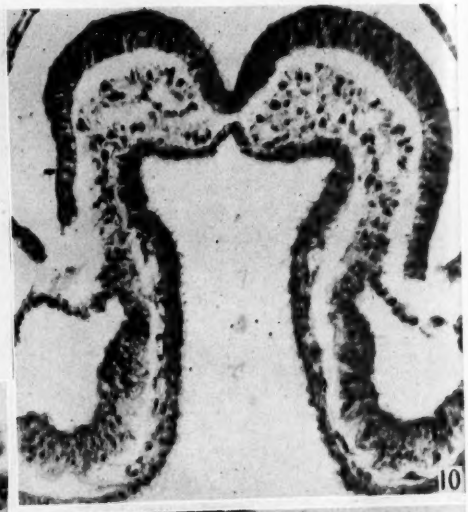
Fig. 15. A high-power view of the heart rudiment shown in fig. 14. The intimate relationship of the endocardium to the entoderm (on the right) is shown, as is also the character of the mesostroma. Magnification ×520, reduced to ×390 for reproduction.

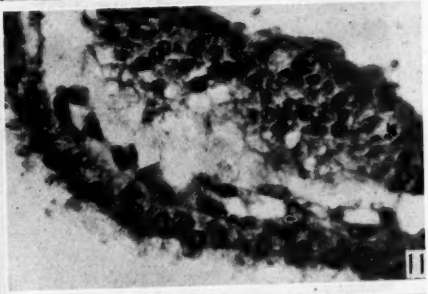
Fig. 16. A section through embryo B 52 A (16 days) just in front of the anterior intestinal portal. The two lateral coelomic channels are separated by a band of tissue—a ventral mesocardium. Magnification ×200, reduced to ×150 for reproduction.

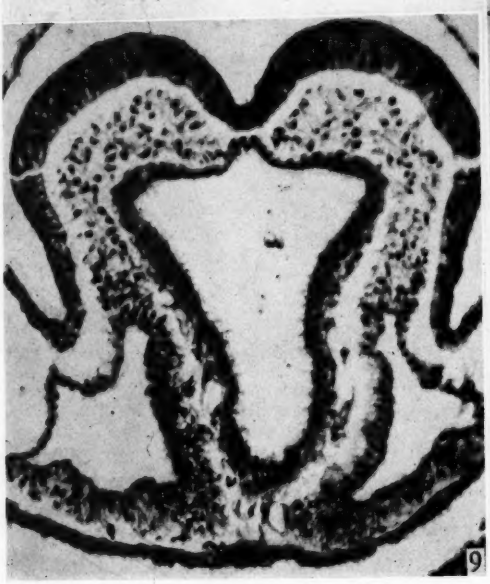



FIELD—THE EARLY DEVELOPMENT OF THE SHEEP HEART

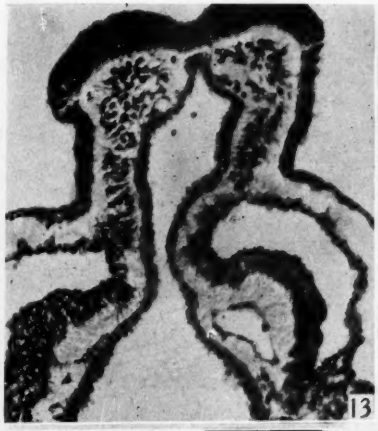


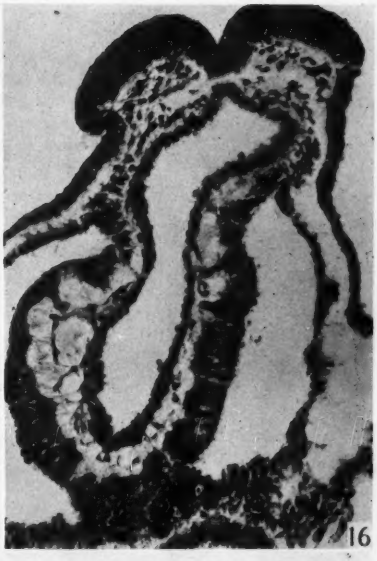



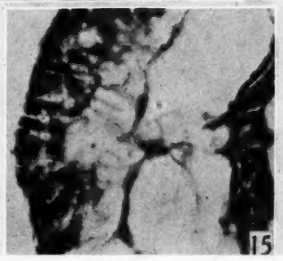


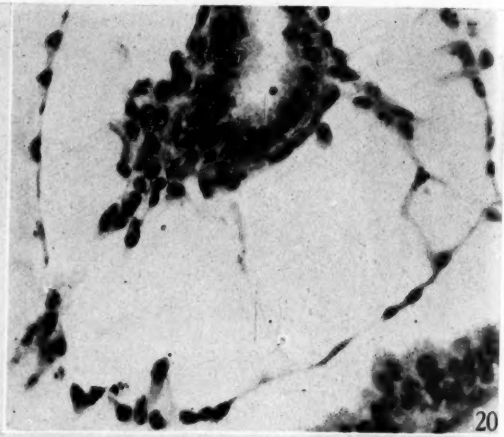
FIELD—THE EARLY DEVELOPMENT OF THE SHEEP HEART

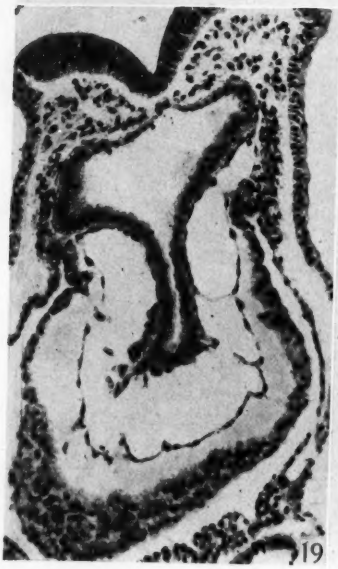





FIELD—The early development of the sheep heart



FIELD—THE EARLY DEVELOPMENT OF THE SHEEP HEART

Fig. 17. A section through embryo B 16R (16 days) taken immediately caudal to the anterior intestinal portal. The few cells between the coelomic folds in the lower part of the field indicate commencing fusion. Well-defined vitelline veins are present on either side, projecting into the lateral coelomic channels. Magnification ×200, reduced to ×150 for reproduction.

PLATE 4

Fig. 18. A section through embryo B 16R (16 days) taken just in front of the anterior intestinal portal. The vitelline veins are in the process of fusing, and below there is a ventral mesocardium. Magnification ×200, reduced to ×150 for reproduction.

Fig. 19. A section through embryo B 16R showing the subpharyngeal heart rudiment at its fullest development in this specimen. Note the wide extent of the 'lake' (cf. Text-fig. 3). Magnification ×200, reduced to ×150 for reproduction.

Fig. 20. A high-power view of part of fig. 19 showing the endocardium in close relation to the foregut floor (above). The remains of contiguous walls of angiocysts project into the lumen of the endocardium like baffle-plates. Magnification ×520, reduced to ×390 for reproduction.

Fig. 21. A section through embryo B 16L (16 days) showing a well-defined ventral mesocardium connecting the heart to the septum transversum. Changes in surface contour of the heart are beginning. Magnification ×200, reduced to ×150 for reproduction.

# CORRELATED CHANGES IN NERVOUS TISSUES IN MALFORMATIONS OF THE CENTRAL NERVOUS SYSTEM

By A. BRODAL, *Anatomical Institute, University of Oslo, Norway*

In the present communication some observations made on a rare type of malformation of the cerebellum will be briefly reported, and the inferences which can be drawn from them in regard to the understanding of the factors which determine the occurrence of correlated changes in the nuclei connected with the malformed part will be discussed.

A perusal of only a limited part of the vast literature dealing with malformations of the central nervous system at once reveals extensive differences and variations within the cases belonging to one type or the other, as e.g. microgyria, anencephalia, cerebellar malformation and others. This is true not only in regard to the intensity of the 'primary' malformation and the accompanying abnormalities in other organs so frequently met with, but also as concerns the accompanying changes within the central nervous system itself. These usually are termed *correlative changes*, indicating thus a causal connexion of some sort or other between them and the principal, major malformation.

The study of cases of this type has, especially in earlier days, been undertaken partly to obtain information concerning the fibre connexions between different regions of the central nervous system, partly to ascertain the factors influencing the orderly development of the central nervous system, the malformations representing, as has often been stated, experiments by nature itself to a certain degree. However, it must be admitted that although certain major features concerning the fibre connexions have been recognized from cases of this type, the conclusions drawn will always be more or less hypothetical, on account of our still very fragmentary knowledge of the factors at work in normal development.* The value of information concerning normal development is likewise restricted, since secondary changes of different kinds may produce extensive disturbances in the picture. Thus very diverging hypotheses have been set forth on this point by different authors.

Experimental and comparative anatomical research has brought forward a considerable mass of evidence, elucidating the factors which are at work in the morphogenesis of the nervous system. Necessarily, however, the experimental studies have for the most part been performed on lower vertebrates, and on systems connecting the central nervous structures with the peripheral organs. In regard to the connexions within the central nervous system, we are thus mainly forced to content ourselves with conclusions of analogy. In single instances only the situation is more favourable, special circumstances allowing more exact conclusions to be drawn directly from findings in higher vertebrates. An opportunity of this sort was afforded by the material forming the basis of the present communication, a strain of mice affected with hereditary hydrocephalus with a partial maldevelopment of the cerebellar vermis.

In his extensive study of cerebellar malformations Brun (1917–18) distinguishes two types of correlative changes in cerebellar malformations, especially of the neo-cerebellum. One type he calls *primary correlative checking of development* ('primär-korrelative Entwicklungshemmung'). In this instance it is assumed that the same noxious factor, which arrests the development of, e.g. the neo-cerebellum, will produce a simultaneous checking of development of those 'subcerebellar' structures which develop previous to or at the same time as the neo-cerebellum. These structures then will be present in such cases, but they will remain at an early foetal stage, eventually, however, presenting further histo-tectonic differentiation.

The second type of correlative changes is named by Brun *secondary correlative checking of development* ('sekundär-korrelative Entwicklungshemmung'). Here the changes in the 'subcerebellar' structures cannot be explained as a consequence of the same noxious factor which has affected the cerebellum, but they must be conceived of as being secondary to it, produced through a repression of the 'morphogenic stimulation' normally exercised by the neo-cerebellum on the structures which are dependent on it (v. Monakow's 'Kleinhirnanteile'). In this case the effect will be limited to structures developing later than the neo-cerebellum, and the histo-tectonic differentiation will fail to take place. As such structures Brun mentions especially the pontine nuclei.

It will appear from his nomenclature that Brun is inclined to explain all malformations as being due to a checking of development. Furthermore, it appears that insufficient emphasis is placed on the possibility of hereditary factors as responsible for the ultimate manifestation of the maldevelopment,

* This has been more fully elaborated in regard to the cerebellum in a previous paper (Brodal, 1940, pp. 127 ff.).

and instead presumable intoxications of the germ or of the foetus are over-emphasized as causal factors. On these points modern conceptions are not in full accord with Brun's rather schematic views, but as this problem falls beyond the scope of the present paper, it will not be treated more fully.

However, Brun has stressed the importance of the time factor in developmental disturbances, especially in the cerebellum. For an understanding of the correlative changes in such instances this is obviously of great value. Although such correlative changes are the rule in cerebellar malformations, other cases are met with where the 'subcerebellar' structures, i.e. the nuclei sending fibres to or receiving fibres from the cerebellum, are quite intact, although parts of the cerebellum are severely malformed or even lacking. For example, Vogt and Astwazaturow's Case I (1912) presented extensive changes in the hemispheres of the cerebellum and anomalies in the vermis, but in spite of this there were no changes in the inferior olive, the brachium conjunctivum or the nucleus ruber, but the pontine grey was devoid of cells. In Verhaart's case (1933) there was a cyst and changed cortical structure in the left hemisphere, but no changes in the nuclei connected with the cerebellum. Obersteiner (1914) describes a brain where most of the vermis as well as the nuclei fastigii were lacking, the two cerebellar hemispheres fusing directly in the midline. Obersteiner states specifically that there was no reduction of the afferent or efferent cerebellar tracts and, although it is not specially mentioned, it is not likely that there had been changes in the nuclei in question.

This lack of correspondence between cerebellar and subcerebellar lesions is usually explained by assuming a certain degree of 'self-differentiation' of the nerve cells. It is imagined that the outgrowing neurites which are prevented from reaching their normal ending place will nevertheless have the capacity of further development, and eventually will take an aberrant course, to end in other regions, preferably in other parts of the same nuclear complex. This assumption appears rather probable, but obviously a process of this type can occur only on the supposition that the nucleus of origin is developed at a time when the major changes are already established in the nucleus or grey matter which is normally the destination of the fibres. If the outgrowing fibres have already reached their ending place or if they have no other way to go, the result will presumably be a secondary affection of the nucleus of origin. (Some observations, however, tend to show that *smaller* structural aberrations, e.g. in the cerebellar cortex, do not interfere with the ingrowth of neurites, e.g. a case described by Miskolczy (1932).)

As far as can be seen from the literature, observations which clearly support the above-mentioned assumption have not been recorded. The study of the foetal stages of the mouse strain with hereditary hydrocephalus, mentioned above, has, however, made possible some conclusions concerning this topic. Below, therefore, a brief survey of the findings in adult and foetal hydrocephalic mice will be given, followed by a discussion of the findings in regard to the problems in question.

The mouse material consists of a strain of the albino house mouse, affected with hereditary hydrocephalus, which has been studied by Professor Kristine Bonnevie, Zoological Laboratory, University of Oslo. The anomaly has been shown to be inherited as a monohybrid recessive, manifesting itself in a hydrocephalus of varying degree (Bonnevie, 1943).

In studies of foetal stages of these mice, Bonnevie (1944) has shown that the first signs of developmental disturbances have occurred already in the blastocyst at or before its implantation in the uterus, manifested first as anomalies in the foetal coverings. Later on the disturbances are transmitted also to the embryo itself, i.e. as an abnormal imbibition of extra-embryonic fluid. The hydrocephalus makes its appearance first on the 12th day of foetal life (Bonnevie, 1943, 1944). In the embryos, as in the adult mice, the degree of hydrocephalus varies from the most extreme dilatation of all the cerebral ventricles to conditions just beyond the normal. The hydrocephalus is constantly accompanied by a splitting of the cerebellum into two more or less complete halves (Fig. 4 (1944)).*

The cerebellar anomalies were made the object of a special study (Brodal, Bonnevie & Harkmark, 1944), and likewise the development of the cerebellar changes during foetal life were studied in embryos from different stages (Bonnevie & Brodal, in the Press).

It was found that, in the least affected adult specimens, the splitting was confined to the caudalmost part of the vermis, e.g. in one cerebellum, shown in Fig. 5 (1944), the lobulus *b*, the uvula, was present as two halves entirely separated from each other; the lobulus *a*, the nodulus, was nearly completely subdivided in two halves, which were only connected rostrally; the most superficial folia of the anterior lobe (lobuli 3 and 4) did not meet in the midline, otherwise the two cerebellar hemispheres were interconnected almost as usual; lobulus *c*, the tuber and pyramis, however, was lacking except for its most caudal folium which was present as two bilateral halves.

In general, the same features were characteristic also of the more severely affected specimens. In some (exemplified by the drawings in Fig. 8 (1944)), the vermis was nearly completely split in two halves, which were only connected at the middle levels by a narrow bridge of cerebellar substance. Also in cerebella affected to this degree the only

* For illustrations the reader is referred to the original papers. In the present paper each figure to which reference is made, is indicated by its number in the original paper and its year of publication (1944 referring to the article by Brodal, Bonnevie & Harkmark, 1945 to that by Brodal).

lobule lacking was the lobulus *c*, with the exception of its caudalmost folium. The hemispheres, the paraflocculi and the flocculi were normal in all these specimens.

At those places where the two cerebellar halves were separated from each other, traces were found of a tiny membrane connecting them. In many of the adult cerebella this membrane had been broken, but from the study of the foetal cerebella it appeared clear that the two halves had originally been connected by a membrane, covered with flattened ependymal cells on the ventricular surface, by the pia mater on the outer surface, and containing as a rule some nerve fibres.

Apart from minor aberrations in the structure of the cerebellar cortex in the vicinity of the median split, some small heterotopias in a few of the cerebella and a probable reduction of the nucleus medialis of the cerebellum, no other anomalies were ascertained. It is to be noted that no abnormalities could be detected in the inferior olive, the pontine nuclei, the nucleus cuneatus externus, the nucleus reticularis lateralis and the vestibular nuclei, in spite of the lack of the largest part of the lobulus *c*, a constant feature in all the investigated hydrocephalic cerebella.

In only one of the microscopically investigated cerebella, which was very severely affected, were there any changes present in the nuclei sending their fibres to the cerebellum. In this specimen (from which the drawings in Fig. 10 (1944) were made), the splitting was complete and the two halves drawn widely apart. Furthermore, the caudal part of the left hemisphere was greatly reduced in size, and part of the nervous substance was replaced by connective tissue. At some places groups of macrophages, partly laden with blood pigment, were present in the connective tissue, and in the cerebellar substance itself other groups of non-pigmented, presumably glial, macrophages were met with. In this specimen there was a considerable reduction of the cell mass of the external cuneate nucleus and the nucleus reticularis on the left side (homolateral to the most affected half of the cerebellum) and in the pontine nuclei, especially the peduncular and lateral grey, and in the inferior olive on the right side. In the inferior olive the medial accessory olive was most reduced, but also the principal olive, especially its caudal parts, and the dorsal accessory olive was poorer in cells than normal.

From the study of the adult cerebella it was concluded that the cerebellar anomalies were a consequence of the hydrocephalus, and this was confirmed by the findings in the foetal material. The hydrocephalus, which sets in on the 12th day of foetal life, will produce a stretching of the cerebellar plate. As this is normally thinnest in its median region, it is to be expected that the attenuation will mainly affect this region, especially at the caudal levels. The attenuation may in extreme cases proceed so far that only a thin membrane is left of the cerebellar plate in the midline, whereas in the lesser affected brains the rostral part of the cerebellar plate will develop nearly normally, thus resulting in a practically normal anterior lobe, combined with a more or less complete splitting of the more posterior parts of the vermis. The quantitative reduction of the cerebellar substance, especially the partial lack of the lobulus *c*, is explained according to the hypothesis of Dow (1940). Dow assumes that a process disturbing the regular development of the cerebellar commissures (commissura lateralis in the flocculo-nodular lobe and commissura cerebelli in the corpus cerebelli) will interfere with the proper migration of neuroblasts from the 'anlagen', and then will affect preponderantly the material destined for the formation of the vermis, whereas the hemispheres will be less affected or entirely normal. As the anterior part of the vermis is the first to develop, an explanation is also obtained of the fact that in all cases of partial lack of the vermis so far reported in the literature the vermis of the anterior lobe is always best preserved.*

Astonishingly similar findings as in these hydrocephalic mice were made in a human case (Brodal, 1945). The patient was a girl, 10 years old. From her first year of life she had presented an enlarged head, which later on continued to grow out of proportion. Clinically and by ventriculography the diagnosis of hydrocephalus was made. In addition to the enlarged head the child presented other symptoms, i.e. reduced vision, an unsteady gait and reduced mental powers. Death occurred some days after a surgical intervention for loosening adhesions in the posterior cranial fossa. At the operation it was disclosed that the caudal part of the cerebellar vermis was lacking.

Macroscopical and microscopical examination post-mortem showed that the cerebellar hemispheres and the vermis of the anterior lobe were nearly normal, only the right tonsil was abnormal in its position and folial pattern. The declive also was almost normal, but the folia caudal to the declive appeared to be lacking. However, on a closer analysis, rudimentary lobules were found which could be identified as the tuber, the pyramis, the uvula and the nodulus. But most of these folia were present as bilateral structures (cf. Figs. 4–12 (1945)). The rudimentary folia representing the tuber, pyramis and uvula were attached to a membrane (*v.m.d.* in Fig. 10 (1945)), which on the ventricular surface was covered with ependyma, on the outer with pia mater and which contained some myelinated fibres. The membrane was attached to the central cerebellar mass on each side, and extended rather far dorsally as a sack-like bulge, but had apparently been damaged at its extreme rostral point during the operation. A velum

* Three cases described by Brun (1918*b*, Cases VI, VII and IX), however, are different in some respects, in so far as in these instances a cleft was found only in the vermis of the anterior lobe. But in all cases there were also rather prominent other anomalies as well in the cerebellum as in other parts of the nervous system (e.g. spina bifida in two instances), which makes it probable that Brun's cases belong to another type of malformation than those treated in this paper.

medullare posterius was also present, continuous both with the flocculi, which presented smaller aberrations in their finer structure, and with the rudimentary folia representing the nodulus, likewise present as small bilateral structures. The nucleus fastigii on the right side was smaller than normal and the nuclei dentati showed slighter structural anomalies.

In this brain also no aberrations from the normal picture in the inferior olive, the pontine grey or other nuclei sending their fibres to the cerebellum could be detected.

An analysis of the findings in this human case thus disclosed striking similarities with the anomalies in the hydrocephalic mouse strain, and it was concluded that the mechanism which was responsible for its origin was of the same nature in both instances, viz. an early developing hydrocephalus, which had impeded the migration of neuroblasts to the future vermis region through attenuation of the cerebellar plate.

The finding in these cases, which is of special interest for the problem to be discussed in the present communication, is the intactness of the nuclei sending their fibres to the cerebellum. Apart from one very severely affected mouse brain, the nuclei in question were intact, in spite of the fact that parts of the cerebellum were always lacking, or clearly underdeveloped in the case of the human brain. Now it must be admitted that a minor reduction of, e.g. the pontine nuclei, the external cuneate nucleus or the nucleus reticularis lateralis might have escaped attention, since it is clearly very difficult to verify a slight reduction in these systems where there exists no clear-cut localization within the projection (cf. Brodal & Jansen, 1943; Brodal, 1941, 1943). In regard to the inferior olive, however, the situation is different. It has long been known from observations made on human material, that the olivo-cerebellar projection system is clearly organized on a localization principle (Henschen, 1907; Holmes & Stewart, 1908; Brouwer & Coenen, 1919; Koster, 1926). By experimental investigations on rabbits and cats by means of a modified Gudden method, it has been possible to ascertain the details of this localization (Brodal, 1940). Each lobule of the cerebellum has its own projection area in the complex of the inferior olive, and also the intra-cerebellar nuclei have their distinct projection areas. The lobulus *c*, which especially concerns us here, receives its fibres in the rabbit from a well-circumscribed area (about one-quarter) of the medial accessory olive, but in the cat from a considerably larger area in correspondence with the more developed lobulus *c* in this animal. It can scarcely be imagined that a reduction of such a large portion of the medial accessory olive will be overlooked, especially when particular attention is paid to it.

From the observations made in the adult mice it was concluded (Brodal *et al.* 1944) that the intactness of the 'subcerebellar' structures, especially of the inferior olive, was probably due to the early development of the cerebellar anomalies, these setting in before the neurites from the inferior olive had reached the cerebellum. The neurites destined for the lacking parts were probably directed to intact regions of the cerebellum. The analysis of the brains of the foetal mice (Bonnevie & Brodal, in the Press) lent active support to this view. A brief survey of the principal findings will elucidate this.

The hydrocephalus, as previously mentioned, makes its appearance on the 12th day of foetal life. On the 11–12th day the cerebellar plate is still undifferentiated into folia. It is considerably thinner in its median part, especially caudally, than in the major lateral parts and there are no signs of fissures or of any cortex. A thin superficial marginal veil, practically free of cells, covers the intermediate mantle layer which, without clear limits, passes into the matrix on the ventricular side of the plate. In the brain stem most of the nuclei of the cranial nerves can be recognized, but there is no inferior olive and no pontine nuclei, and the corpus ponto-bulbare, which provides the material for the pontine nuclei and the inferior olive (Essick, 1912) has just begun its mitotic activity.

The first clear-cut indication of vigorous cellular proliferation in the ponto-bulbar body is encountered at the 12th day stage. Approximately on the 13th day, the olivary migration along the lateral border of the medulla oblongata makes its appearance, and on the 13–14th day the cells from this migration are found to accumulate at the site of the future inferior olive. Not until approximately the 17th day are the separate subdivisions of the olive recognized, but at this time many of the cells still appear immature. The olivary migration subsides about the 17–18th day.

The pontine migration, and the formation of the pontine nuclei, which begins about the 14th day lags a little behind the olivary migration and the differentiation of the inferior olive, although the delay is not nearly so pronounced as in man.*

The first indication of lobulation in the cerebellum of the normal mouse is encountered on the 17th day. Then the fissura postero-lateralis of Larsell (Larsell & Dow, 1935) makes its appearance, closely followed by the fissura prima. The cortical differentiation begins on the 14th day as a clear-cut embryonic granular layer separated from an inner granular layer by a fibre-rich narrow zone, and still at the 18th day stage the Purkinje cells are not fully developed. The separate lobules of the vermis can be identified from the 18th day stage, but at birth the lobulation of the cerebellum and likewise the cortical differentiation are not yet completed.

In the abnormal foetal mice the hydrocephalic dilatation with the ensuing attenuation of the cerebellar plate, beginning on the 12th day, leads to a more or less severe

* This can be explained partly as a consequence of the much more rapid development of the brain in the mouse, partly owing to the greater development and complexity of this nuclear mass in man as compared with the mouse.

reduction of the median part of the plate. Finally, the two halves are connected partially or in their entire rostrocaudal extension by a thin membrane only. The ultimate degree of the cerebellar changes, however, as mentioned previously, is highly variable. The changes taking place in the cerebellar plate, however, clearly do not interfere with the processes leading to the formation of the pontine nuclei and the inferior olive. At all stages these, like the ponto-bulbar body and the migrations arising from it, develop exactly as in the normal specimens, and finally they present a completely normal picture in the adult hydrocephalic mice. The process affecting the cerebellar plate appears to be entirely mechanical.

It thus appears that in the abnormal mice the hydrocephalus, and the attenuation of the cerebellar plate which is the consequence of it, sets in earlier than the full activity of the corpus pontobulbare. The subdivisions of the inferior olive are differentiated first at the 17th day, approximately 5 days after the beginning of the damage to the cerebellar plate, and at the time when the first signs of lobulation appear, only one day before the subdivision of the vermis into its different lobules is clearly recognized. Unfortunately, the sections at my disposal were not fibre stained.* However, judging from the findings in the sections employed (van Gieson stain, fixed in Bouin's fluid) it appears safe to conclude that the fibres from the inferior olive cannot have reached the cerebellum during the lapse of one day, the interval between the recognition of the separate subdivisions of the olive and the separation of the vermis into its lobules. Of course, it cannot be strictly denied that some of the cells of the olive at this time might have rather well-developed neurites, but certainly a half of them have not, as many cells in the olive are still distinctly undifferentiated. The same may be said of the accessory olives and the pontine nuclei.

Assuming now that at least the major portion of the fibres from the olive have not reached the future vermis region at the 18th day, when the cerebellar changes are clearly established, it is likely that the fibres destined for this part of the cerebellum, especially for the constantly lacking major part of the lobulus *c*, will be directed to other parts of the cerebellar cortex. They will find an aberrant ending place in the cerebellar cortex, and at this point establish qualitative normal connexions. In this manner no changes will appear in the inferior olive. Similar considerations can obviously be applied to the pons and the ponto-cerebellar fibres.

The assumption was made above that the proliferating cerebellar cortex will attract the incoming cerebellopetal fibres. Without embarking on a detailed discussion of the factors concerned in the morphogenesis of the brain some data of special interest will be briefly mentioned.* Thus it is learned from the experimental investigations made especially on amphibia by Coghill (1924), Detwiler (1928) and Burr (1932) that proliferating centres have the capacity of attracting ingrowing fibres. That the reverse also holds good, ingrowing fibres having the capacity of stimulating the activity of a proliferating centre may also be mentioned (Burr, 1920; May & Detwiler, 1925).

However, it is obvious that these factors alone cannot explain the orderly morphogenesis of the nervous system. The investigations of Coghill, Detwiler and others clearly demonstrate that there are other forces at work. Obviously the proliferation of the cellular masses is to a great extent determined by 'intrinsic dynamic forces', of whatever sort they may be, especially early in development. Thus as concerns the medulla spinalis of amphibia Coghill (1933, p. 345) states: 'There is, therefore, an intrinsic dynamic substratum of progressive development in the region irrespective of peripheral, afferent nerves; and there is evidence... that the intrinsic dynamic substratum persists after the establishment of the afferent nerve roots.' That the development of the inferior olive takes its usual course in the hydrocephalic mice in spite of the considerable changes taking place simultaneously in the cerebellum is a fact confirming this conception. In this instance conditions are also especially favourable for such a judgement, as the factor responsible for the cerebellar malformation is clearly purely mechanical, produced by the hydrocephalus, and can scarcely be thought to affect the ponto-bulbar body to any considerable extent.

It will be recalled that in one case only (Fig. 10, 1944) were clear-cut changes found in the pons as well as in the inferior olive, preponderantly in the parts corresponding to the lacking parts of the cerebellum. This, however, probably does not make untenable the hypothesis set forth above. It cannot be denied that the severe change in one cerebellar hemisphere in this case had taken place later and was associated with haemorrhage and secondary ingrowth of mesenchyme (cf. Brodal *et al.* 1944). On the other hand, the affection has from the beginning been severe, and the reduction of the one hemisphere can be dated back to an early foetal stage. But when in this brain the fibres from the normally developing inferior olive have reached the cerebellum, it can be assumed that the cortical areas left have not been extensive enough to receive them all. On this account some of them have fallen the victim of atrophy, which in the foetal brain is followed by a total disintegration with a rapid clearing up by the neuroglia. That the damage to the olive is found preponderantly in the regions corresponding to the lacking areas is in accordance with the considerations made previously.

* The mouse strain died out before the microscopical study was begun.

* Excellent reviews of these problems have been published by Detwiler (1926) and Kappers (1934).

A mechanism similar to that in the hydrocephalic mice will also reasonably explain the lack of changes in the inferior olive and the pons in the human case briefly reported above. In the analysis of the findings in that case (Brodal, 1945) it was regarded as probable from a comparison with the normal development of the human cerebellum as described by Hochstetter (1929) that the hydrocephalus had set in when the embryo had a length of approximately 43 mm. According to Essick (1912) the first cells at the site of the future inferior olive in man can be recognized before the 30 mm. stage. But not until approximately the 143 mm. stage is the pattern of the olive established with its typical subdivisions. The characteristic foldings, however, are not yet visible. Thus also in this instance the hydrocephalus must be assumed to have arisen considerably earlier than the full development of the inferior olive and still longer prior to the pontine nuclei. When in some of the previously published cases of the same type (defective development of the vermis) changes were observed in the inferior olive, e.g. in the cases of Lyssenkow (1931) and Castrillón (1932), and Dow (1940), they might be due to a comparatively later setting in of the hydrocephalus. Besides, in Lyssenkow's and Dow's cases the hemispheres also were partially affected.

The chief interest in the cerebellar malformations discussed in this paper is the lack of correlative changes. It appears safe to state that in the case of the hydrocephalic mice it has been possible to prove that this lack can be explained by the very early development of the primary cerebellar malformation, some time in advance of the full development of the nuclei in connexion with it. It appears plausible that similar conditions may be responsible for the lack of the usual correlative changes now and then observed also in other types of cerebral malformations.

## SUMMARY AND CONCLUSIONS

The findings in the brains of a strain of mice affected with a hereditary hydrocephalus are reported. The most prominent change apart from the hydrocephalus was a partial or complete splitting of the cerebellar vermis with lack of the lobulus *c*, except its caudalmost folium. The nuclei sending fibres to the cerebellum were intact. A closely similar human case is also briefly described. In both instances there is reason to assume that the cerebellar anomaly has been produced by an attenuation of the cerebellar plate caused by the hydrocephalus.

From an analysis of normal and hydrocephalic mouse embryos it appears that the hydrocephalus sets in on the 12th day of foetal life, at a stage when the activity of the ponto-bulbar body is just beginning. The separate subdivisions of the inferior olive cannot be recognized until 5 days later, and even at this stage many of its cells are not fully differentiated. The lobulation of the vermis can be recognized approximately one day later.

Based on these findings, the hypothesis is set forth that the lack of correlative changes in the inferior olive (and the pons) can be explained in the following manner: At the time when the neurites from the cells of the olive reach the cerebellum, the malformation is already fully developed (as abnormal embryos from this stage also show). The ingrowing neurites which do not find their normal ending place are directed instead to the remaining intact regions, thus preventing the occurrence of atrophy and changes in the inferior olive, pons and other nuclei.

A mechanism of the type described in this material might give a valid explanation in other instances of malformation, where the usual correlative changes are lacking.

## REFERENCES

Bonnevie, K. (1943). *Norske Vid.-Akad. Skr.* I. Mat.-Nat. Kl. no. 4.
Bonnevie, K. (1944). *Norske Vid.-Akad. Skr.* I. Mat.-Nat. Kl. no. 10.
Bonnevie, K. & Brodal, A. (1946). *Norske Vid.-Akad. Skr.* I. Mat.-Nat. Kl. (in the Press).
Brodal, A. (1940). *Z. Neurol.* **169**, 1.
Brodal, A. (1941). *Z. Neurol.* **171**, 167.
Brodal, A. (1943). *Acta Psychiat. Neurol. Kbh.* **18**, 171.
Brodal, A. (1945). *Norske Vid.-Akad. Skr.* I, Mat.-Nat. Kl. no. 3.
Brodal, A., Bonnevie, K. & Harkmark, W. (1944). *Norske Vid.-Akad. Skr.* I. Mat.-Nat. Kl. no. 8.
Brodal, A. & Jansen, J. (1943). *Norske Vid.-Akad. Skr.* I. Mat.-Nat. Kl. no. 10.
Brouwer, B. & Coenen, L. (1919). *J. Psychol. Neurol., Lpz.*, **25**, 52.
Brun, R. (1917). *Schweiz. Arch. Neurol.* **1**, 51.
Brun, R. (1918*a*). *Schweiz. Arch. Neurol.* **2**, 48.
Brun, R. (1918*b*). *Schweiz. Arch. Neurol.* **3**, 13.
Burr, H. S. (1920). *J. exp. Zool.* **30**, 159.
Burr, H. S. (1932). *J. comp. Neurol.* **56**, 347.
Castrillón, H. A. (1932). *Z. Neurol.* **144**, 113.
Coghill, G. E. (1924). *J. comp. Neurol.* **37**, 71.
Coghill, G. E. (1933). *J. comp. Neurol.* **57**, 327.
Detwiler, S. R. (1924). *J. comp. Neurol.* **37**, 1.
Detwiler, S. R. (1926). *Quart. Rev. Biol.* **1**, 61.
Detwiler, S. R. (1928). *J. exp. Zool.* **51**, 1.
Dow, R. S. (1940). *J. comp. Neurol.* **72**, 569.
Essick, C. R. (1912). *Amer. J. Anat.* **13**, 25.
Henschen, F. Jr. (1907). *Z. klin. Med.* **63**, 115.
Hochstetter, F. (1929). *Beiträge zur Entwicklungsgeschichte des menschlichen Gehirns.* Leipzig u. Wien: Deuticke.
Holmes, G. & Stewart, T. G. (1908). *Brain*, **31**, 125.
Kappers, C. U. A. (1934). *Irish J. med. Sci.* Sept.
Koster, S. (1926). *Acta Psychiat. Neurol. Kbh.* **1**, 47.
Larsell, O. (1934). *Arch. Neurol. Psychiat., Chicago*, **31**, 373.
Larsell, O. (1937). *Arch. Neurol. Psychiat., Chicago*, **38**, 580.
Larsell, O. & Dow, R. S. (1935). *J. comp. Neurol.* **62**, 443.
Lyssenkow, N. K. (1931). *Virchows Arch.* **280**, 611.
May, R. M. & Detwiler, S. R. (1925). *J. exp. Zool.* **43**, 83.
Miskolczy, D. (1932). *Arch. Psychiat. Nervenkr.* **93**, 596.
Obersteiner, H. (1914). *Arb. Neurol. Inst. Wiener. Univ.* **21**, 124.
Verhaart, W. J. C. (1933). *Acta psychiat. Neurol. Kbh.* **8**, 691.
Vogt, H. & Astwazaturow, M. (1912). *Arch. Psychiat. Nervenkr.* **51**, 75.

# MOVEMENTS OF THE FIRST RIB

By R. WHEELER HAINES, *Department of Anatomy, St Thomas's Hospital Medical School*

## INTRODUCTION

The most varied opinions as to the movements of the first ribs have been held by various workers, and are still to be found in modern text-books. In the cadaver the first rib undoubtedly moves with the rest when the lungs are inflated or the sternum drawn upwards, and Magendie (1831, p. 378) described this rib as moving more than other members of the series. Sibson (1846) in a careful study of the dissected cadaver found that when the thorax was artificially inflated the first rib moved with the others, keeping parallel to them, towards a more horizontal position, and Thane's (1892, fig. 187) figure shows the sternum rising as a whole and the first rib moving with the others. But Haller (1758) had found that for a moderate inspiration the lower end of the sternum moved forwards more than the upper end, and this was taken by later writers to indicate that the manubrium, and so the ribs attached to it, could move very little under any circumstances. Reid (1852, p. 334), for instance, mentions Magendie's conclusion only to contradict it, and Allen Thomson (1876, p. 312), though he admitted that 'on the prepared skeleton, by raising and depressing the sternum the ribs may be moved upwards and downwards nearly parallel to one another; the first rib moving as freely as the others', suggested that 'during life several causes combine to make the first rib more fixed than those that follow: as for example, the weight of the upper extremity, and the strain of the intercostal muscles and the ribs beneath'.

This uncertainty as to the amount of movement of the first rib has persisted in spite of the demonstration of considerable movement of the manubrium sterni in deep inspiration by outline tracings (Hutchinson, 1852), photographic records (Hasse, 1901), radiographs (Macklin, 1925), and kymographs (Weber, 1936) and figures illustrating the movement (Keith, 1909, fig. 2), and is reflected for instance in Blair's (1943, p. 341) statement that in inspiration 'the upper border of the sternum is only slightly raised and is carried forward hardly at all'.

The mechanism of the first rib is still less understood. Sibson's (1846) figures, made with the aid of a drawing machine, showed it quite clearly rising from a more oblique to a more horizontal position during inflation of the lungs, and at the same time turning so that its outer border came to look more laterally and less downwards. Several authors whose results have been tabulated by Fick (1911, p. 143) have published measurements showing that the axis about which the first rib moves is oblique, though less so than those of the succeeding ribs. Henke (1863, p. 79) described the axis as passing outwards and at the same time upwards and backwards, but Landerer (1881) could not confirm this, for he found the axis directed horizontally outwards and backwards, with no upward component.

Estimations of the degree of obliquity of the axis (the angle between the axis and a line drawn horizontally outwards) are also inconsistent. Henke and Meissner gave high figures (42 and 36°, tables of Fick, 1911, p. 143), Trendelenburg, Volkmann (same tables), Landerer and Fick low figures (9–16°). Later authors have followed Braune (1888) in stating that the axis of the first rib is nearly transverse. Further, the shortness of the first costal cartilages seems to preclude any movement similar to the lateral displacement of the lower ribs, so that even those authors who, like Weber (1936), are prepared to allow the first rib a considerable range of movement usually restrict that movement to a simple lid-like raising and lowering of the thoracic inlet as a whole. Thane's (1892, p. 161) statement that 'in the first rib the axis is nearly transverse, and thus while a considerable degree of elevation is permitted the eversion is but slight', and Blair's (1943, p. 341) that 'in the upper ribs, where the axis is almost transverse, the lateral movement is slight or absent' are fair samples of anatomical opinion.

Yet a cursory examination of a preparation of the first rib *in situ* is sufficient to show that the movement is not lid-like and the axis is not transverse. Each rib is seen to move round an oblique axis, in inspiration the lateral border moves further from the midline, and the directions in which the surfaces of the rib face are remarkably altered. This paper is concerned with a detailed study of the mechanisms involved in the cadaver and in the living subject.

## ANATOMICAL PREPARATIONS

*Axis of movement.* In a wet preparation of the first costal ring, from a man about 60 years of age, a steel knitting needle was fixed to the rib by plasticine and its position adjusted till it pointed the same way whether the rib was raised or lowered (method of Henke, 1863 and Landerer, 1881). The fact that this was possible showed that the rib was turning about a stationary axis and the movement was a simple rotation. The axis was found to pass through the head, along the neck and through the tubercle, outwards, backwards and somewhat upwards, as stated by Henke (1863). It was very far from the

simple horizontal axis so often described, and the axes of opposite ribs met in the fore part of the body of the first thoracic vertebra at an angle of 110°, so that the obliquity, as defined above, was 35°. In the other ribs the axes pointed downwards rather than upwards, except for the second where the axis was directed nearly horizontally.

*Range of movement.* The preparation with needles fixed in the positions of the two axes could now be mounted in a drawing frame in convenient positions, and accurate orthoscopic projections made with the ribs raised and lowered. For purposes of measurement direct vertical (Fig. 2), anterior (Fig. 4) and lateral views are the most convenient, for the vertical, lateral and anterior displacements of the ribs can be read off from them directly, while a view taken directly along the line of the axis (Fig. 1) gives the total movement at the costo-chondral junction and the angular movement of the rib about the axis. The mechanism is however better understood from oblique views (Fig. 3), for these bring out the peculiar movements about the two axes very clearly.

In the specimen illustrated the right rib turned through an angle of 24°. The distance from the axis of movement to the centre of the costo-chondral junction was 75 mm., so that the anterior end of the rib was displaced through 31 mm., and the sternum was displaced through a corresponding distance. When the specimen was mounted as nearly as could be judged in the position it occupied in life, the 31 mm. of total displacement was found to be made up of 28 mm. of vertical and 14 mm. of anterior movement. The maximum distance between the lateral borders of the two ribs increased by 9 mm., but the distance between the costo-chondral junctions of the two sides, 85 mm., was unchanged.

Comparing the first rib with the others the actual displacement of its anterior end was less, for the anterior end of the 7th rib of the same individual, for example, moved through 45 mm., but owing to the great length of the rib this displacement was brought about by an angular movement of 14° only, so that so far as angular movement was concerned the first rib moved more than the others, and Magendie's (1831) statement on this point was confirmed.

*Trajectory.* Since the axis of movement, if we ignore small displacements, remains stationary, it follows that each point on the rib must describe an arc of a circle about the axis as the rib turns, and a view along the axis will show the anterior end moving along such an arc (Fig. 1). Other views show the circular trajectory projected as an ellipse, and it is convenient to draw in the ellipse before considering the mechanism of the rib as a whole. A wire *AB* (Fig. 2) is fixed so that it lies along the straight line from the costo-chondral junction *B* to the axis *OAX*, and meets the axis at a right angle *OAB*. In practice since the point *A* is embedded in the bone of the first thoracic vertebra the wire cannot actually reach the axis, though it can point in its direction. A second wire *AC* is fixed so as to pass from the axis at the same point as the first, and again at a right angle *OAC* to the axis, but in the plane in which the drawing is being made, i.e. the plane of the paper. A third wire *AD* stands at right angles both to *AC* and to the axis, so that *AO*, *AC* and *AD* might lie along adjacent margins of a cube

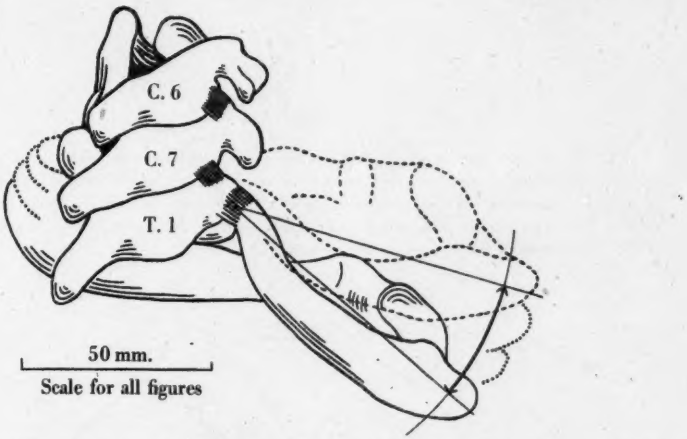



Fig. 1. Orthoscopic tracings of the first rib in inspiration and expiration in a wet preparation, taken looking directly along the axis, showing the true angular movement of the rib about the axis.

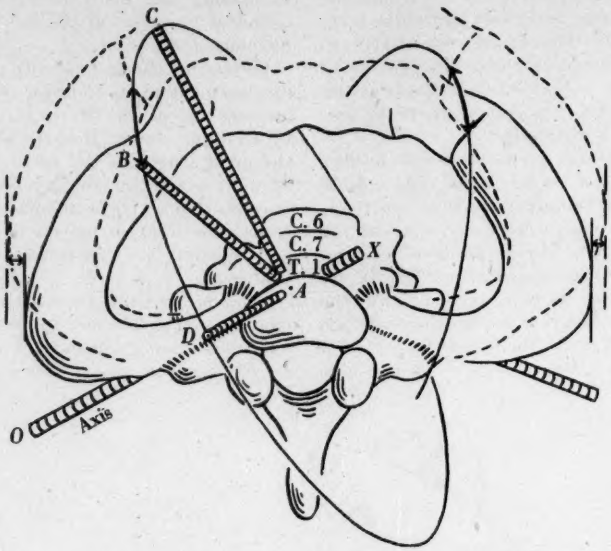



Fig. 2. Orthoscopic tracings taken from below looking vertically upwards, showing movement of the first ribs round the oblique axes, the forward movement of the cartilages and sternum, and the lateral displacements of the shafts of the ribs. *AB*, *AC*, *AD* indicate the positions of wires used in the construction of the theoretical trajectory of the costo-chondral junction.

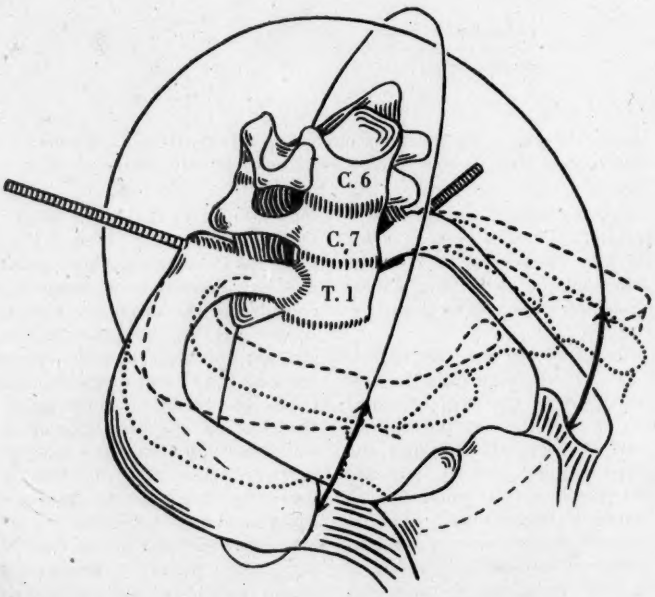



Fig. 3. Oblique view of the thoracic inlet.

whose corner was at *A*. The lengths of the wires *AC* and *AD* are now adjusted so that each is equal to the distance *AB*, and their positions are traced on to the projection drawing. *AB*, *AC* and *AD* represent three radii of the circle which would be traced by the costo-chondral junction if it turned round the axis *AOX*, and if they have been correctly placed the wires will all lie in the same oblique plane, the plane of the circle. Further, *AD* will, in the drawing, be superposed to *AOX*.

On a separate sheet of paper a family of ellipses, the projections of a circle of radius equal to the length of *AB*, are drawn by ordinary geometrical procedure. The particular curve whose centre is at *A*, whose long axis lies along *AC*, and which passes through the point *D* is now traced through on to the drawing, and gives the presumed trajectory of the costo-chondral junction. The curves of Figs. 2 and 3 were drawn in this way; they are projections of circles of different diameters, 150 mm. on the right, 140 mm. on the left, because the lengths of the ribs differed on the two sides (quite a common condition). We now have in the same drawing tracings showing the actual positions of the ribs as they are raised and lowered, and the curves which would be traced out by the anterior ends of the ribs if they described perfect circles round fixed axes. The actual and predicted positions are seen to correspond closely, and the proposition that the ribs move about axes which are placed obliquely and not horizontally is confirmed.

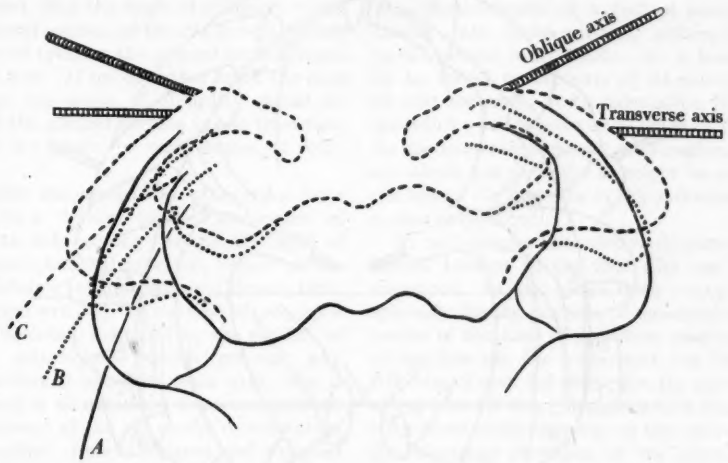



Fig. 4. Antero-posterior view showing movement round the oblique (broken line) and transverse (dotted line) axes. *A* indicates the position of the lung in expiration, *B* the position it would occupy if the ribs were rotated round the transverse axis, and *C* the position it does occupy when they rotate round the oblique axes.

*Adjustment of the trajectory.* One obvious difficulty stands in the way of the acceptance of the oblique axis. If the axes were directed horizontally outwards the costo-chondral junctions would always be at the same distance from each other, but with the axes placed obliquely they must tend to move relatively to each other as the ribs are raised and lowered, and the short horizontally placed first costal cartilages would prevent any appreciable movement. This difficulty has probably caused the rejection of the hypothesis of the oblique axis by workers who have considered it; certainly Weber (1936) in his monograph on kymography of the chest says that the shortness and rigidity of the cartilages restricts the movement of the thoracic inlet to a simple raising and lowering of the inlet as a whole.

Fig. 5 shows the trajectories of the anterior ends of a pair of isolated ribs mounted so as to turn round oblique axes. As they are raised from their lowest point on the elliptical trajectories at *AA′* they rapidly draw away from each other so that at *BB′* they are much further apart, but as they pass towards the horizontal position their rate of separation falls off, so that at *CC′* they are separating from each other very slowly and at *DD′* their relative movement is zero. Beyond this they move closer together, slowly at *EE′* and increasingly rapidly beyond. Now the actual range of movement in life corresponds to the part of the trajectory between *CC′* and *EE′* where the relative movement is at a minimum near the point of reversal. The actual trajectories enforced by the attachments to the

unyielding costal cartilages are represented by the lines (straight in projection) *CE* and *C'E'*, but the difference between the curved paths traced by the isolated ribs and the paths enforced by the costal cartilages is but 1·5 mm. at the maximum. The length of the rib in this specimen is 65 mm., that of the neck, 25 mm., so that a movement of about 0·6 mm. at the tubercle or the head is sufficient to adjust the rib to its course. Furthermore, the maximum adjustment is required when the rib is near the middle of its range of movement, at the time when the laxity of the ligaments is greatest.

So the anterior ends of the ribs turn through just those parts of the trajectories around the oblique axes where their lateral displacements will be least. The directions of the necks of the ribs, along which the axes of the movement pass, must be adjusted so that the range of movement corresponds to the region of minimal lateral displacement. The axes must pass upwards as well as outwards, and so the heads of the ribs must lie at lower levels than their tubercles. It is this requirement that necessitates the sharp bend at the junction of the neck of the first rib with the shaft, and the atypical articulation of the head with but one vertebra.

Fick (1911) suggested quite a different mechanism. He described the axes of the ribs as oblique but horizontal, so that they passed from the heads outwards and backwards but not at the same time upwards. This would lead to a considerable separation of the anterior ends as the ribs turned during inspiration, and he suggested that they did in fact separate, stretching the costal cartilages as they did so. He quoted evidence of the extensibility of cartilaginous tissue, and presented a model in which the costal cartilages were represented by extensible springs. Fick did not, however, quote any measurements in support of his views, and in the material at my disposal there is no measurable lateral displacement of the costo-chondral junctions.

*Direction of the pleural surfaces.* If the right and left ribs moved as a single unit around a horizontally placed transverse axis, they would suffer no relative displacement, and there would be no gain in the distance between their outer borders during inspiration, but in fact there is a considerable gain, which no doubt plays a part in the expansion of the lungs. At the same time there is a reorientation of the pleural surface. *A* in Fig. 4 shows the position at extreme expiration, *B* the change in position brought about by tilting the thoracic inlet till the sternum occupies its inspiratory position without displacing the ribs relatively to each other, that is using the supposed horizontal axis. The pleural surfaces of the ribs now face more outwards than before, and would allow the lung to expand to the position indicated. In *C*, however, the ribs are shown in the position enforced on them by the true axis. Not only is the vertical and lateral displacement of the middle of the shaft greater than it would

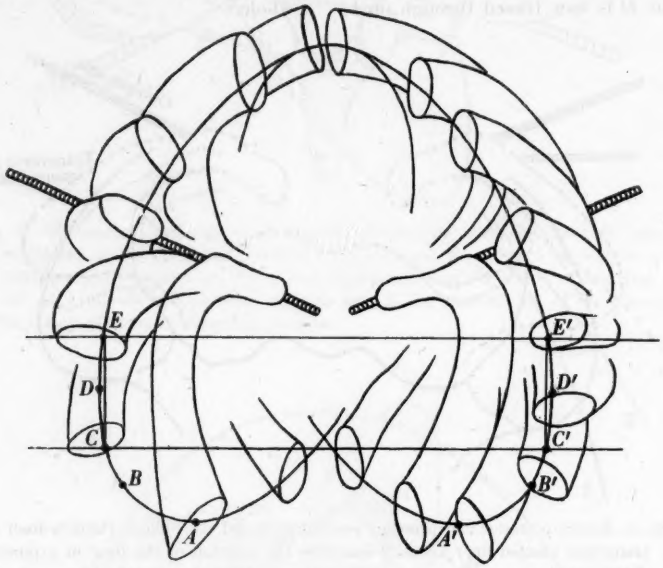



Fig. 5. Antero-posterior tracings from dry bones turning round the oblique axes showing the trajectories of the costo-chondral junctions. In life the range from *C* to *E* and *C'* to *E'* is the part of the curves utilized.

be if the movement were about a horizontal axis, but the pleural surface faces more downwards and less inwards, and the expansion of the lung is correspondingly increased.

Geometrically when a plane surface rotates about an axis the change in direction in which the plane faces depends on how the surface is set on the axis. The change is zero if the axis is set vertically to the plane, and increases with the angle of obliquity of the axis to the vertical (very nearly as the sine of the angle for small rotations), and the change is maximal when the axis is parallel to the plane (here for each degree of rotation about the axis the direction in which the plane faces changes by a full degree). In the first rib the maximum cannot be realized for the precise direction of the axis is determined by other mechanical requirements already discussed. But the angle of obliquity of the axis to the pleural surface of the rib is over 60°, so for each degree of rotation the pleural surface turns through about 0·9°. If on the other hand the axis were transverse the angle of obliquity would be about 45°, and the pleural surface would then turn through about 0·7° only for each degree of rotation.

Rather similar movements in other ribs have been ascribed to a 'bucket handle' movement of the rib about an axis passing through the head of the rib and costo-chondral junction, forced on the rib by the 'resistance' of the sternum (Meyer, 1885, and many modern writers). In the first rib any such movement is absolutely excluded by the rigidity of the vertebral attachments which prevent any measurable movement about such an axis. Nor is such a movement at all necessary to account for the lateral displacement of the rib or the reorientation of its pleural surface. Indeed isolated ribs mounted so as to turn about axes passing along their necks describe the wing-like movements just as well as ligamentous preparations with the sternum preserved (Fig. 5).

The observations and deductions have been repeated on several occasions from dead bodies of both sexes, including a young man of 22 known to have been in the best of health up to the time of his death from head injuries. The material includes specimens with the whole of the thoracic cage intact, and others with the manubrium sawn through below the attachment of the first costal cartilages. The detailed measurements vary from one individual to another but the same general features are found in all the normal material examined.

## THE LIVING SUBJECT

In life the first rib is difficult to observe, but in two subjects, both female, the outer borders of the two ribs could be felt as they moved beneath the skin, and in one of these subjects, a physiotherapist who had specialized in respiratory movements, the first ribs could be seen moving relatively to each other.

In the preparation shown in Figs. 1–4 the sternum was found to move 28 mm. upwards and 14 mm. forwards and it was important to check whether this range of movement could occur in life. The measurement is rather difficult to make, for, as is well known, most subjects tend to straighten the thoracic part of the curvature of the vertebral column in deep inspiration, and this exaggerates the movement of the sternum. Ransome (1873) fixed the back by seating his subjects on a straight-backed chair, and recorded the thoracic movements with an ingenious machine which automatically analysed the displacements of a button pressed on to the thorax into their vertical, antero-posterior and medio-lateral components. In a healthy male of 45 he found movements of 34 mm. upwards and 22 mm. forwards, and a calculation from his eleven records for males gives an average of 21 and 15 mm. So the movements in my preparation, though they are above the average, as might be expected from the size of the first ribs in this individual, are by no means exceptional.

In radiographs the relative displacements of the lateral borders of the first ribs can be seen and measured. In two series from young males taken specially for the purpose of measurement, with the centre of the cone of radiation passing at the level of the first rib, the movement was 10 and 11 mm. Allowing 2 mm. for distortion the movements were about 8 and 9 mm., measurements which agree well with those made directly on the cadaver. Further, the changing direction of the pleural surface is clearly seen in the radiographs. Similar displacements of the lateral borders can be seen in the stock radiographs used for teaching, and in many of those figured in the literature (e.g. Macklin, 1925), but when the cone of radiation is centred below the level of the first rib the change in direction of the pleural surfaces is not demonstrated so clearly. Lateral views are disappointing, as the soft tissues are too opaque to allow satisfactory films to be obtained.

Taken alone observations on the living subject would tell little of the mechanism, but so far as they go they confirm the conclusion that each rib is turning round its own oblique axis.

## CALCIFICATION OF THE COSTAL CARTILAGES

The contradictions in the various accounts of the movements of the thoracic inlet, and the prevalence

of the statement that the axis of the first rib is transverse or nearly so may be due to the study of specimens with reduced mobility of the first costal cartilage. In such a specimen, taken from a relatively young woman of 45, in which the cartilages were completely calcified and there was no recognizable movement between the first rib and the sternum, the axis was transverse, the displacement of the sternum was limited to 12 mm., and the ribs, cartilages and sternum moved round the axis as a single unit. A more detailed study showed that the common axis passed through the two costo-transverse joints, whose ligaments were strong, but fell well behind the costo-vertebral joints, whose ligaments had become very lax, so as to allow the head of the rib to slip upwards and downwards on the body of the vertebra.

Other specimens showed conditions intermediate between the extremely oblique type of axis described earlier and the transverse type, with 20° and 14° of obliquity. Here there was some limitation of movement, particularly of the lateral displacements of the ribs, and the costal cartilages were found to be partially calcified. It seems probable that similar specimens have been examined by those authors who have described the axes as nearly transverse, for they form the bulk of the material available to anatomists.


## SUMMARY

1. The movements of the first ribs have been studied in the cadaver and in the living subject.

2. The first ribs do not turn round a transverse axis common to both ribs, but each rib turns round an oblique axis passing outwards, backwards and upwards along its neck.

3. The angular movement about the axis is greater in the first rib than in the others of the series.

4. The shaft of the rib is displaced upwards and laterally in inspiration and the pleural surface comes to look more directly downwards.

5. The axes are so placed that the costo-chondral junctions can remain at the same distance from each other as the ribs are raised. The direction of the neck relative to the shaft and the atypical articulation of the first rib with but one vertebra are determined by this requirement.

6. The rotation round oblique axes leads to a greater expansion of the lungs than would be brought about by a rotation round transverse axes.

7. Calcification of the first costal cartilages leads to a limited movement of the ribs and sternum as a single unit about a transverse axis.


My thanks are due to Prof. A. B. Appleton for the anatomical material, and to Dr J. W. McLaren for the radiographs.

## REFERENCES


BLAIR, D. M. (1943). 'Arthrology.' In Cunningham's *Text-book of Anatomy*, 8th ed. Oxford Univ. Press.

BRAUNE, W. (1888). *Arch. Anat. Physiol., Lpz.*, p. 304.

FICK, R. (1911). *Handb. Anat. Mensch. Gelenke*, Th. III. Jena: Fischer.

HALLER, A. (1758). *Mémoire sur plusieurs Phénomènes Importantes de la Respiration*. Lausanne. Quoted by Reid (1852).

HASSE, C. (1901). *Arch. Anat. Physiol., Lpz.*, p. 273.

HENKE, P. J. W. (1863). *Handbuch der Anatomie und Mechanik der Gelenke*. Leipzig and Heidelberg: Winter.

HUTCHINSON, J. (1852). 'Thorax.' In Todd's *Cyclop. Anat. Physiol.* **4**, 1016. London: Longman, Brown, Green, Longmans and Roberts.

KEITH, A. (1909). 'The mechanism of respiration in man.' In Leonard Hill, *Further Advances in Physiology*, p. 182. London: Arnold.

LANDERER, A. (1881). *Arch. Anat. Physiol., Lpz.*, p. 272.

MACKLIN, C. C. (1925). *Amer. J. Anat.* **35**, 303.

MAGENDIE, F. (1831). *An Elementary Compendium of Physiology for the use of Students*. Tr. E. Milligan, 4th ed. Edinburgh: Carfrae.

v. MEYER, H. (1885). *Arch. Anat. Physiol., Lpz.*, p. 253.

RANSOME, A. (1873). *Med.-chir. Trans.* **56**, 82.

REID, J. (1852). 'Respiration.' In Todd's *Cyclop. Anat. Physiol.* **4**, 325. London: Longman, Brown, Green, Longmans and Roberts.

SIBSON, F. (1846). *Philos. Trans.* **136**, 501.

THANE, G. D. (1892). 'Arthrology.' In Quain's *Elements of Anatomy*, 10th ed., **2**, pt. I. London: Longmans, Green and Co.

THOMSON, A. (1876). 'Myology.' In Quain's *Elements of Anatomy*, 8th ed., **1**. London: Longmans, Green and Co.

WEBER, H. H. (1936). 'Die normal Atmung.' In Stumpf, Weber & Weltz, *Röntgenkymograpische Bewegungslehre innerer Organe*, p. 242. Leipzig: Thieme.

# TERMINAL DEGENERATION IN THE FRONTAL CORTEX OF THE RABBIT FOLLOWING THE INTERRUPTION OF AFFERENT FIBRES

By P. GLEES, A. MEYER and M. MEYER, *Departments of Anatomy and Physiology, Oxford, and Neuropathology Department, Central Pathological Laboratory, London County Mental Health Services*

In their recent communication on boutons terminaux in the cerebral cortex, Meyer & Meyer (1945) reported on biopsy specimens of the frontal lobes. They observed numerous annular or bulb-like structures, morphologically similar to the boutons terminaux commonly seen in the spinal cord. The question arose in their study, whether these structures are indeed terminals comparable to those present in the normal spinal cord or whether they represent pathological changes in very fine unmyelinated nerve fibres. The frontal lobe material was removed during operations for intracranial tumours and the possibility could not be eliminated that even parts of the cortex distant from the neoplasm might have been affected by pathological changes or by the well-known effect in biopsy material of rapid fixation. Further investigations of two senile brains reported in the paper by Meyer & Meyer showed a similar wealth of terminal rings in the cortex and the latter observation suggested strongly the possibility of a pathological increase of bouton-like structures in old age or perhaps a greater affinity of normal boutons for silver stains, making terminal rings more readily visible.

These observations initiated the present experimental approach for studying terminal nervous structures within the frontal cortex. In the experiments here described, most of the afferent fibre connexions to the frontal lobe were severed by an incision almost separating the frontal lobe from the rest of the cerebral hemisphere. A detailed histological study of the cortex was made after different time intervals. One main source of these afferent fibres to the frontal cortex must be the thalamus (Le Gros Clark & Boggon, 1933) and since this pathway may be of significance in connexion with the operation of frontal leucotomy in the human brain, the study of the frontal cortex after its experimental interruption assumes some importance.

Distinct changes following human leucotomy have so far been reported only in the medial or dorso-medial nucleus of the thalamus (Freeman & Watts, 1942, 1944; Meyer & Beck, 1945). These pathological changes are caused by retrograde cell changes following the interruption of the thalamo-frontal fibres. Freeman & Watts (1942, 1944) in their communications deny that there is an appreciable effect on the frontal lobe after leucotomy, judged by the Nissl and myelin pictures. However, quite recently Meyer & Beck (1945) found in the third layer of the human frontal lobe a slight but definite reduction of nerve cells, suggesting that efferent fibre connexions also from the frontal lobe had been severed by prefrontal leucotomy.

It must be stressed that hitherto no studies have been made with the silver method and it is thus probable that the changes occurring in thinly or non-myelinated fibres in the frontal cortex have remained undetected. The present study is, therefore, mainly concerned with the degenerative changes of terminals which occur in the frontal cortex after cutting the thalamo-frontal fibres, which form the majority of the afferent fibres. The periods of survival of the experimental material were necessarily short in order to detect the early changes in the process of degeneration of the unmyelinated fibres. These early changes can be demonstrated in the region of synapses and incidentally may also give information about synaptic relations in the cortical layers.

## MATERIAL AND METHODS

Twenty-eight mature rabbits were used in the series of experiments. The skull was opened under aseptic conditions and after cutting the dura the posterior boundaries of the frontal lobes were mapped out by identification of the motor cortex with faradic stimulation. A deep incision was made with a v. Graefe knife posterior to the motor cortex. Haemorrhage was usually stopped without difficulty by gentle pressure with a saline swab. Sixteen out of the twenty-eight animals were used for the present paper. The remaining animals were excluded, either because the incision was not deep enough or because of post-operative infection. Survival times ranged from 5 min. to 28 days.

The animals were killed with an overdose of nembutal and the brains fixed in 5% formol-saline. After 24 hr. the brains were sliced sagittally, to ensure penetration by the formalin. Sagittal sections were cut of both hemispheres, the normal side serving as control. To demonstrate nerve fibres and terminals, two silver impregnation methods were used, the Bielschowsky method and the method described below. For comparison with silver impregnated slides the Nissl method and the Marchi reaction of degenerating myelin were employed.

One of us (Glees, 1946) has developed a silver

technique which has proved to be very useful in the demonstration of degenerating unmyelinated nerve fibres and terminals. The technical procedure is as follows:

(1) Fix blocks of tissue for at least 5 days in 5% formol-saline and cut on the freezing microtome at 12–15 $\mu$.

(2) Wash sections well in distilled water and place for 24 hr. in 50% alcohol with 5–10 drops of concentrated ammonia.

(3) Wash in distilled water and place into 10% silver nitrate for 24 hr. or longer, up to 5 days if necessary until sections are brown.

(4) Without washing, reduce in several changes of 10% formalin made up with tap water (commercial formalin taken as 100% solution).

(5) Place sections into the following ammoniacal silver solution: 10 c.c. absolute alcohol, 10 c.c. 20% silver nitrate, adding ammonia until the precipitate so formed is redissolved. Sometimes a few more drops of ammonia have to be added to obtain the desired results. Here the sections remain for $\frac{1}{2}$–1 min.

(6) Repeat reduction in formalin, step 4.

(7) Wash twice in distilled water and fix in 5% hypo.

(8) Wash well, dehydrate in graded alcohols, clear in creosote, and mount.

## OBSERVATIONS

### A. *Examination of the normal frontal cortex of the rabbit*

The finer histological structure of the cerebral cortex has been dealt with in admirable detail by von Bonin (1944). In this recent account the whole functional aspect of the terminating fibres within the cortex has been considered and the morphology of the cortical synapse has been discussed. Von Bonin's paper makes it unnecessary to give a full description of the normal cortex in this investigation. We limit our description to those observations made on normal silver stained sections which are necessary for the comparison of the experimental material.

The silver stained sections of the rabbit's cortex reveal in principle the same complexity of structure as the human cortex (Pl. 3, fig. 12). Characteristic fibre elements form the basic pattern namely the bundles of radiate fibres (Pl. 3, fig. 13), single running oblique fibres and the horizontal plexus formations (lines of Baillarger). According to Polyak's Marchi studies of degenerated thalamo-cortical fibres (1932) the terminating ascending fibres have an oblique or tortuous course. These incoming fibres can easily be identified in our silver stained preparations. The normal preparations show an even greater density of the plexus of the horizontal fibre striation than myelin sections and reveal the pericellular plexus around individual pyramidal cells (Pl. 3, fig. 15).

The study, however, of this pericellular plexus reveals very few bouton-like structures similar to the boutons terminaux in the spinal cord (Pl. 3, figs. 14, 16) (see also Cajal, 1911). These ring formations of the frontal cortex are either in contact with the dendrites of the cortical cells or in relation to the cell body. While examining the normal frontal cortex for the presence of bouton-like structures, a fair number of ring-like endings were found to be regularly present in the lateral nucleus of the thalamus (Pl. 2, fig. 11) and the superior colliculus. These fine rings are in contact with the cell body, thus representing axo-somatic synapses. Since no particular mention of boutons in the thalamus or superior colliculus has so far been reported in the literature, it is appropriate to record these observations in this paper. So far, no attempt has been made to ascertain the terminal degeneration of fronto-thalamic fibres in the dorso-medial nucleus of the thalamus. In order to do this coronal sectioning would have been necessary which was thought to be less suitable for the purpose of this paper. It is intended to study the terminal degeneration in the dorso-medial nucleus in a new series of experiments.

### B. *Experimental material*

To follow the degeneration of nerve fibres and their terminals for a maximal distance, sagittal sections through the whole extent of one hemisphere were cut and stained by the methods described above. In these sagittal sections three different zones of degeneration could be distinguished:

(1) The region of the incision.

(2) The degeneration of the severed fibre tracts of the frontal lobe.

(3) A part of the frontal cortex furthest removed from the incision in order to exclude traumatic influence as much as possible. [The conditions found in 11 of the 16 animals employed are described below.]

*R.T.D.* 14. Death 5 min. after operation. Histological examination of the area of incision shows clean cut fibres. No formation of rings or retraction bulbs is visible.

*R.T.D.* 25. Death 24 hr. after operation. The reaction of the fibres near the cut is well marked. A profusion of rings and retraction bulbs immediately adjacent to the cut surface is present. In the cortex close to the zone of injury thickened endings on nerve cells can be demonstrated. The degeneration of fibres is decreasing in intensity the further away from the lesion. In the frontal cortex, apart from very few fine disintegrating fibres, no conspicuous pathological changes are apparent. Some rings are seen on nerve cells, but no more than normally

present. The finest fibres seen in disintegration are confined chiefly to the 3rd layer.

*R.T.D.* 17. Death 2 days after operation. Severe degenerative reaction of nerve fibres immediately adjacent to the cut. Cajal's retraction rings predominate in the cortical layers and the bulbs in the white matter (Pl. 1, figs. 1, 2). The finer fibres seem to form rings whilst thicker fibres form bulbs. The degeneration of pathways from the lesion has hardly increased in extent compared to that found in R.T.D. 25. The only sign of degeneration more distant from the lesion is occasional swelling of fibres (Pl. 1, fig. 3). Further away still, fibres are entirely normal. In the tangential layer of the frontal cortex of areas furthest removed from the incision, numerous fibres are beaded and some disintegrating. In the 3rd and 4th layer, the beginning of terminal degeneration and beading of many fine fibres can be observed. Thickened black stained bulbs or large fibrillated structures (Pl. 1, fig. 4) resembling degenerating boutons terminaux, are seen on nerve cells or their dendrites. Very fine rings are seen at the surface of nerve cells (Pl. 1, fig. 5). The appearance of fine rings and widespread beading of fibres is similar to the histological findings in biopsy material described by Meyer & Meyer (1945). Only the fine fibres are beaded (Pl. 1, fig. 6), while the thicker fibre bundles seem to be normal. In the deeper cortical layers there is little evidence of terminal degeneration, although occasionally beading of fine fibres can be found.

*R.T.D.* 18. Death 2 days after operation. There is present considerable oedema of the whole area from which bone has been removed. The frontal pole and occipital and lateral part of the cortex are not oedematous. It is of interest to note that no increase of fibre or terminal degeneration in the oedematous area is to be found, apart from a large haemorrhagic area in which many rings and even retraction bulbs around nerve cells are seen. Apart from this, the terminal degeneration within the oedematous area does not differ from that found within the non-oedematous area and is identical with R.T.D. 17.

*R.T.D.* 19. Death 3 days after operation. There is marked reaction of the nerve fibres to the incision. Retraction bulbs, clubs and rings immediately adjacent to the cut are numerous. When following up the fibre tracts leading to the frontal cortex a slight increase of degeneration is seen. Whereas in R.T.D. 17 the only sign of change is an occasional swelling of a nerve fibre, in this case swelling and ballooning can be frequently observed. These signs of beginning degeneration are still confined to the vicinity of the incision. The part of the fibre tracts close to the frontal cortex is apparently normal.

In the cortical layers itself, however, the fibres of the pericellular plexus are in the initial phase of disintegration. Thickened terminals are seen lying freely in the nervous tissues or on nerve cells and dendrites (Pl. 2, fig. 10). The terminal degeneration has increased in intensity compared with R.T.D. 17.

*R.T.D.* 9. Death 4 days after operation. Retraction rings and bulbs do not begin in the area immediately adjacent to the cut, which is lined by a small area of debris. Most fibres are in a state of degeneration for quite a considerable distance from the lesion. However, in the region close to the frontal cortex, nerve fibres are as yet intact. Considerable degeneration of finer fibres can be observed in all cortical layers, and the finest fibres in particular show all signs of disintegration, while the larger fibres are still intact (Pl. 2, fig. 7). Beading of some of the finer fibres is still present and precedes the granular disintegration. In the 3rd and 4th cortical layers, a great number of thickened rings and bulbs are in contact with nerve cells (Pl. 2, figs. 8, 9) and their dendrites. Frequently whole groups of black staining rings can be detected when carefully sought. The fibre bundles, which enter the cortex through its deeper layers, are relatively intact up to the point of branching into terminal fibres. When these fibres are followed towards the incision, the signs of degeneration gradually increase until the area of incision with all the characteristic signs, described above, is reached.

*R.T.D.* 11 *and* 20. Death 7 and 8 days after operation. The gap caused by the cut is filled with debris. The reaction to the incision is marked in these cases by numerous astrocytes framing the area of incision in the cortex and the white matter. Only the large retraction bulbs are still present and very few rings can be detected. The fibre tracts have undergone marked degeneration and only few fibres appear to be entirely normal. Swelling and ballooning of fibres is frequent and numerous astrocytes in the course of the tracts are found. The destruction of the fibres can be followed from the incision into the frontal cortex. In the latter, numerous astrocytes are found to be scattered in all cortical layers and the previously described signs of degeneration of the finest unmyelinated terminals are present in all layers. Close examination also reveals a slight rarefaction in fine fibres mainly in the 3rd and 4th layers.

*R.T.D.* 23, 15 *and* 13. The animals killed 14, 21 and 28 days after the operation can be described together as the results vary only in the intensity of degeneration. In the area of incision, few rings and retraction bulbs are still seen two weeks after the operation and any such signs of fibre reaction to the lesion have entirely disappeared after 3 weeks. The area of debris has increased correspondingly. Degeneration of the fibre tracts has progressed still further until 4 weeks after the operation almost all the thicker fibres are disintegrating. Astrocytes are very prominent throughout the whole of the

frontal lobe after 2 weeks and later and the debris of fine and thicker fibres is visible in every layer of the frontal cortex. In later cases a distinct rarefaction of fine fibres is present, particularly in the superficial layers of the frontal cortex. The remaining fine fibres are entirely normal, suggesting that they are either intrinsic fibres of the frontal cortex itself or originate from fibre tracts which have not been affected by the operation.

Nissl staining of frozen sections was carried out in all cases. This technique shows clearly the cellular glio-mesodermal reaction in the area of operation extending for some distance in an anterior and posterior direction. Even that part of the frontal cortex farthest removed from the cut is not quite normal in its cytological appearance. After 4 days (R.T.D. 9) the cyto-architecture of the superficial layers is somewhat obscured by the beginning glial proliferation. In later stages, after 2 (R.T.D. 23), 3 (R.T.D. 15) and 4 (R.T.D. 13) weeks the astrocytic proliferation in the frontal cortex increases further and is accompanied by a slight reduction in the number of nerve cells in layer 3 and possibly 4. Many nerve cells appear to be shrunken while others display a vacuolic degeneration not unlike that known as 'water change'. Typical retrograde cell degeneration is found to be infrequent in our material. According to Brodal (1939) the retrograde degeneration is far more easily recognized in experiments on new-born rabbits than in mature rabbits.

The interpretation of these histological findings in the Nissl picture in the small brain of the rabbit is difficult also owing to the vicinity of the cut to the examined area and the peculiar appearance of nerve cells in normal animal brains after rapid fixation (Meyer & Meyer, 1945). It is interesting, however, to compare the changes in the 3rd layer of the frontal cortex of the rabbit with the loss of nerve cells in the 3rd layer of the prefrontal cortex, described in the human brain after leucotomy (Meyer & Beck, 1945).

## DISCUSSION

Histological examination of the normal frontal cortex of the rabbit reveals comparatively few structures similar to the boutons terminaux of the spinal cord, in contrast to the lateral nucleus of the thalamus and superior colliculus where ring-like axo-somatic and axo-dendritic synapses can frequently be found. However, our experimental studies of the early stages of fibre degeneration within the frontal cortex show types of pathological changes undergone by the finest fibres which closely resemble the boutonal type of degeneration. It has been pointed out previously (Glees, 1944) that the difficulty of observing boutons in the normal state is in itself no contra-indication for the study of 'terminal degeneration'. This term includes the boutonal type of degeneration and the degeneration of the apparently free terminals of the pericellular plexus. If the fibre tract ending, either in boutons or in free terminals, is severed, the terminal arborizations show quite distinct pathological changes which bear a close relation to the time which has elapsed between the production of the experimental lesion and the death of the animal. Furthermore, the present experiments show that the first visible changes after injuring ascending fibres to the cortex, apart from the area of experimental lesions, occur in the terminal ramifications of these fibres around the cortical cells. In the first stages of degeneration ring-like structures become more numerous, and fine swellings, in the form of black dots, are found in close contact with the surface of the cortical cell. These observations clearly demonstrate the great value of studying this type of terminal degeneration: for this is the only morphological method for demonstrating the actual termination of fibres, and in this way a knowledge of the localization of the individual synapse and the extent of a synaptic layer can be obtained.

While on the 4th day the terminal degeneration is to be seen particularly well in the 3rd and 4th layers, it is not limited to them, but can be found in varying degrees in all layers. This increased area of degeneration reaching beyond the 3rd and 4th layers may be explained by the fact that an ascending axon not only aborizes in the 3rd and 4th layer, but also gives off collaterals to deeper layers (Cajal, 1911; Lorente de No, 1939). The same factors apply to the degeneration of collaterals as to the degeneration of the main arborizations. However, the degeneration of the main arborizations is more conspicuous and observed more clearly due to the density of the fine arborizations of the terminating axons. The axons themselves clearly show conspicuous signs of degeneration only in the neighbourhood of the actual injury. After this initial stage the degenerative changes rapidly spread to involve the axons in their entire length.

This sequence of degenerative changes has not been observed in the peripheral nervous system by Young (1945) who favours the view that 'all stages of degeneration appear with approximately equal frequency in all parts of the peripheral nerve'. It remains to be seen whether this indicates a difference between the peripheral and central nervous system. The present authors feel that more detailed studies of the pathological conditions within the central nervous system will be necessary to settle this problem.

The fact that ring-like structures appear to become more numerous in certain pathological conditions is in itself no conclusive proof that they are not normally present, as has been pointed out by

Meyer & Meyer (1945). Pre-existing terminal rings and bulbs apparently stain more readily with a silver technique during degeneration. It is conceivable that some chronic processes affecting the brain, such as a slowly growing tumour, senile changes or inherent properties of biopsy material, may have an effect on the synaptic apparatus and may find its morphological expression in an increased visibility of terminal rings. It may further be possible that this swelling of terminals can persist for quite a long time before a further breakdown of the fibres themselves, whose terminal endings are now apparent, occurs.

As has been stated above, terminal degeneration can be observed far from the actual zone of injury and can be studied before the intermediate part of the nerve fibres shows conspicuous signs of degeneration. We would like to stress this fact because the criticism might be made that the delicate changes of the terminal arborizations were due to the immediate effect of the injury. However, the type of terminal degeneration and its selective occurrence in certain cortical layers during the initial stages and the sequence of progressive degeneration in relation to the period of survival is identical with previous findings where direct traumatic influences could certainly be excluded (Glees, 1942). We therefore feel justified in classifying the observed changes as true terminal degeneration. Our experiments show that the interruption of the greater part of the fibres entering the frontal lobe causes a distinct degeneration of terminating fibres mainly in the 3rd and 4th layers, indicating that these are the main receptive layers. It is interesting to recall that the slight loss of nerve cells described by Meyer & Beck in their post mortem findings of human cases after prefrontal leucotomy was also most marked in the 3rd layer of the prefrontal cortex. It is not yet possible to decide whether this loss is due to trans-neuronal degeneration after degeneration of afferent nerve fibres or to the interruption of efferent fronto-thalamic or other tracts. Further investigations in this direction are of some importance as they may help to elucidate the anatomical mechanism of psychological improvement after prefrontal leucotomy.

## SUMMARY

An experimental investigation has been undertaken in the rabbit in order to trace the terminal degeneration of unmyelinated nerve fibres which occurs in the frontal cortex after cutting afferent fibre tracts. A new silver technique, suitable for staining degenerating terminals has been used. Although the operation was more extensive, it simulated to a certain extent the conditions of prefrontal leucotomy.

Terminal degeneration was particularly marked in the 3rd and 4th layers of the prefrontal cortex and corresponds in degree and appearance to the length of survival. In the earlier stages many bouton-like bulbs and rings were seen and their significance has been discussed.

With a selective operative technique the method of tracing terminal degeneration should prove useful in the investigation and localization of poorly myelinated or unmyelinated tracts.

We wish to thank Professor Le Gros Clark for his interest and the facilities offered in his laboratory, and the London County Council for an expenses grant given to one of us (M.M.).

## REFERENCES

v. Bonin, G. (1944). *The Precentral Motor Cortex.* University of Illinois Press.

Brodal, A. (1939). *Z. ges. Neurol. Psychiat.* **166**, 646.

Cajal, Ramon y (1911). *Histologie du système nerveux.* Paris.

Freeman, W. & Watts, J. W. (1942). *Psychosurgery.* Springfield and Baltimore.

Freeman, W. & Watts, J. W. (1944). *J. ment. Sci.* **90**, 532.

Glees, P. (1942). *J. Anat., Lond.*, **76**, 313.

Glees, P. (1944). *J. Anat., Lond.*, **78**, 47.

Glees, P. (1946). *J. Neuropath. exp. Neurol.* **5**, 54.

Le Gros Clark, W. E. & Boggon, R. H. (1933). *J. Anat., Lond.*, **67**, 215.

Meyer, A. & Beck, E. (1945). *J. ment. Sci.* **91**, 411.

Meyer, A. & Meyer, M. (1945). *J. Anat., Lond.*, **79**, 180.

Lorente de No, R. (1939). *J. Neurophysiol.* **2**, 402.

Polyak, S. (1932). *The Main Afferent Fiber Systems of the Cerebral Cortex in Primates.* Berkeley: University of California Press.

Young, J. Z. (1945). *The History of the Shape of a Nerve Fibre.* Essays on Growth and Form. Oxford: Clarendon Press.

## EXPLANATION OF PLATES

Apart from Figs. 4 and 5, which are Bielschowsky staining, the method of P.G. as described briefly in the text has been used. (See Glees 1946 for a fuller report.)

### PLATE 1

Fig. 1. Nerve fibres ending in rings of minute and larger size in the grey matter close to the cut. 2 days after operation. ×1600.

Fig. 2. Nerve fibres in the white matter near the region of the cut showing Cajal's retraction bulbs. 2 days after operation. ×1600.

Fig. 3. The only sign of fibre degeneration distant from the lesion are occasional swellings. 2 days after operation. ×1600.

Figs. 4, 5. Enlarged terminal rings on the surface of nerve cells in the frontal cortex. 2 days after operation. ×1600.

Fig. 6. Beading of fine nerve fibres in their course through the grey matter of the frontal cortex. 2 days after operation. ×1600.

### PLATE 2

Fig. 7. Disintegration of fine nerve fibres within the 3rd and 4th layers of the frontal cortex. 4 days after operation. ×800.

Figs. 8, 9. Thickened bulb-like terminals in contact with nerve cells of the frontal cortex. 4 days after operation. ×1600.

Fig. 10. An enlarged terminal which is the ending of a fine fibre is in contact with a nerve cell of the frontal cortex. 3 days after operation. ×1200.

Fig. 11. Normal terminals in contact with a nerve cell of the lateral thalamic nucleus. ×1600.

### PLATE 3

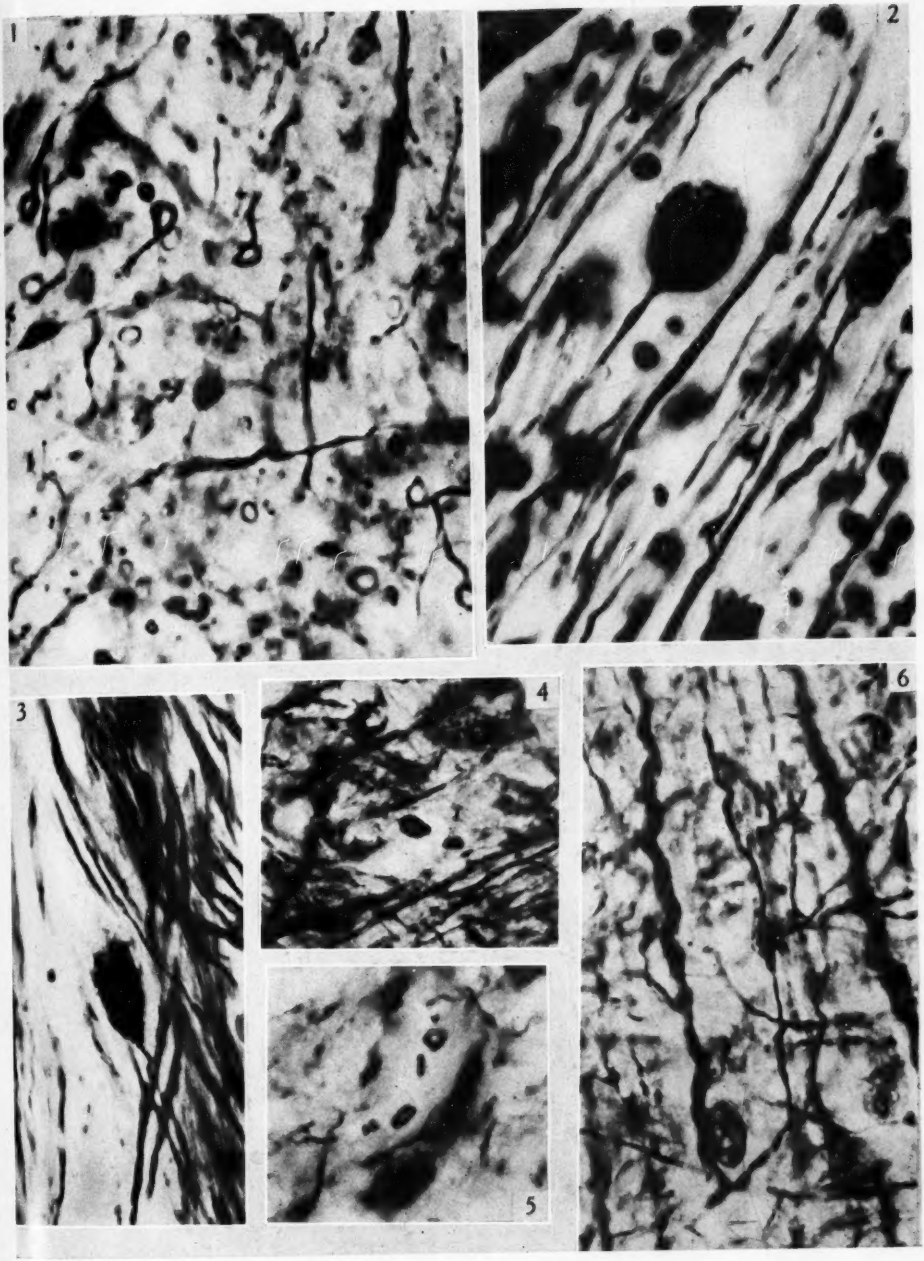
Fig. 12. The normal frontal cortex seen under low power. ×60.

Fig. 13. The basic fibre pattern of the cortex, note the radiate bundles arranged at regular distances and the obliquely running fibres. ×320.

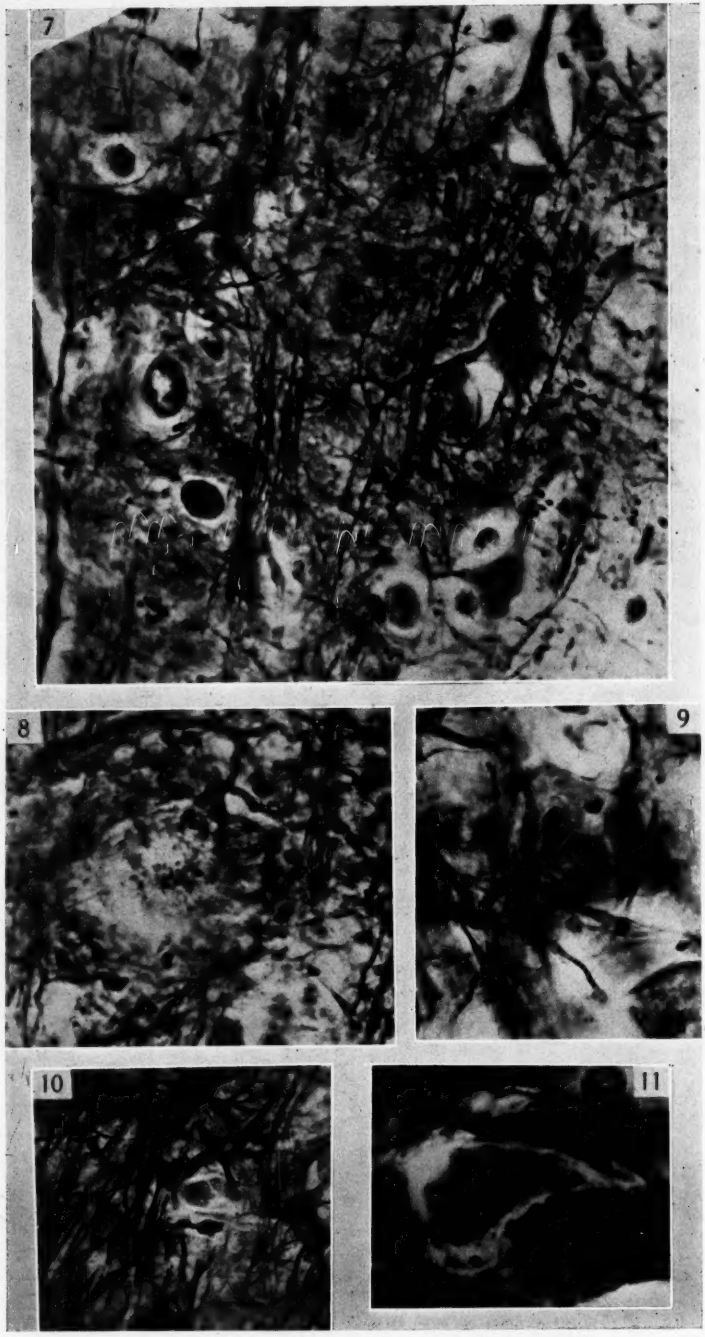
Fig. 14. Occasionally a terminal ring can be detected in contact with a nerve cell. ×800.

Fig. 15. Pericellular fibres around a pyramidal cell of the 4th layer. ×800.

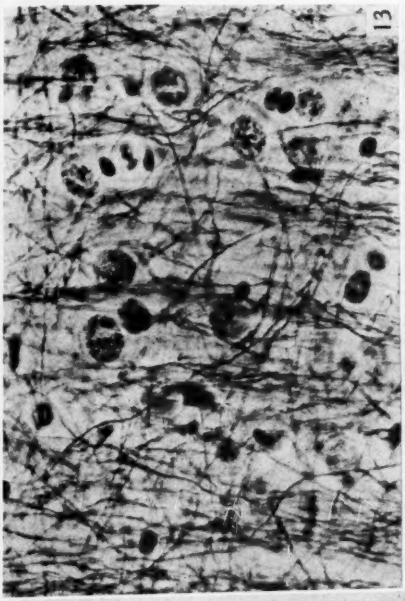
Fig. 16. Pericellular fibres in the 3rd layer. ×800.

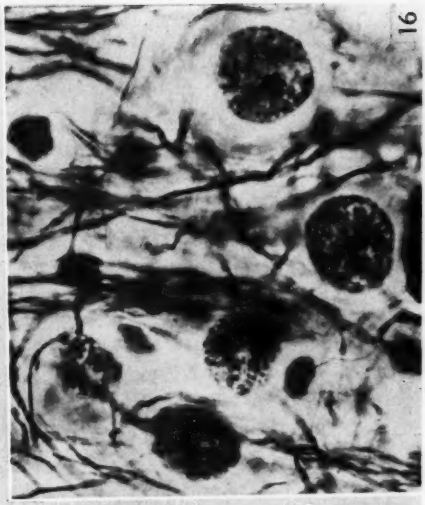


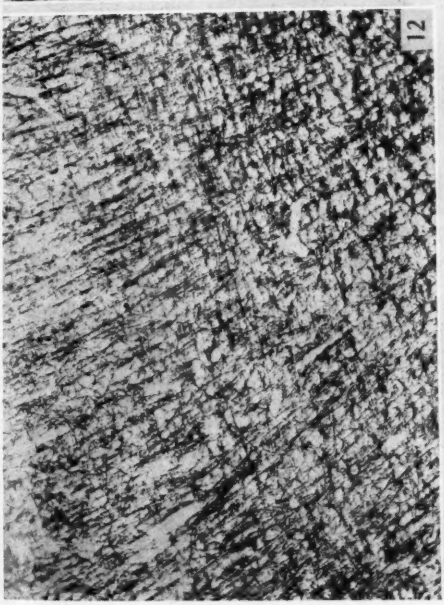

GLEES—TERMINAL DEGENERATION IN THE FRONTAL CORTEX OF THE RABBIT

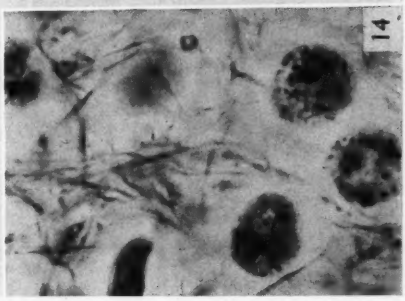



GLEES—TERMINAL DEGENERATION IN THE FRONTAL CORTEX OF THE RABBIT






GLEES—TERMINAL DEGENERATION IN THE FRONTAL CORTEX OF THE RABBIT

UNIV. OF MICH.

# THE PRESCAPULAR LYMPH NODE OF THE OX AND ITS RELATION TO LYMPHATIC DRAINAGE OF THE SKIN

By W. M. HENDERSON, *Research Station (Foot-and-Mouth Disease Committee), Pirbright, Surrey*

## INTRODUCTION

It has already been reported that an intracutaneous injection is almost entirely intralymphatic, that an injection of the skin of the neck of the ox is quickly followed by the appearance of the inoculum in the prescapular lymph node and, moreover, that only one particular part of the node is involved following intracutaneous injection of one particular site (Henderson, 1944). In the ox the prescapular lymph node is a large single structure of variable size and weight lying close to the cranial border of the supraspinatus muscle and covered laterally by the omo-transversarius and the brachiocephalicus muscles. Its length is 6–12 cm., breadth 3–4 cm. and thickness 1–2 cm. The mean weight of 142 nodes was 31·4 g., the standard deviation of the series being ±6·7 g. The long axis is roughly parallel to the long axis of the scapula. The dorsal pole is smaller and more pointed than the ventral pole. The hilum extends for about three-quarters of the length of the medial surface lying nearer the cranial than the caudal border. Sisson & Grossman (1938) describe the prescapular lymph node of the ox as receiving afferent vessels from the skin of the neck, shoulder, part of the ventral and lateral surfaces of the thorax, and the thoracic limb; from the muscles of the shoulder girdle, and from the external scapular muscles; from the tendons of the muscles of the forearm and digit and the fascia of the forearm; from the joints of the carpus and digit. The observations recorded in this paper have been confined to drainage from the skin in an attempt to define the areas of the skin that drain to particular portions of the node.

This lymph node of the ox is of importance in veterinary medicine as it can be palpated in the living animal, it is easily accessible for examination in the course of meat inspection in the dead animal and it receives afferent vessels from the part of the body most commonly used in the ox for intracutaneous and subcutaneous injections.

## METHOD

Devon steers were injected intracutaneously at various sites on the neck, shoulder, thorax and forelimb. The inoculum used was either india ink or a 1 per cent. aqueous solution of trypan blue. The dose injected was usually 3–5 c.c., but in some instances doses of 0·1–10 c.c. provided information about drainage when this was not the primary purpose of the injection. The cattle were killed at intervals after injection varying from a few seconds to 24 hr. and the prescapular lymph node removed. The portion of the node stained by the inoculum was recorded and in many cases the whole route of drainage from the injected site to the node was studied.

## RESULTS

### *Lymphatic drainage of the skin to the prescapular lymph node as demonstrated by the intracutaneous injection of india ink*

A dissection of the neck of the ox following the intracutaneous injection of about 3 c.c. of india ink shows that the inoculum penetrates the interstices of the corium of the skin forming a deeply stained area about 3 cm. in diameter, readily seen on the internal surface of the skin. Much of this stained area is composed of ink-filled lymphatics of the rich plexus of the deeper layers of the corium which drains into a number of larger lymph vessels lying in close apposition to the skin. These larger vessels contain valves which permit of lymph-flow only in the direction of the regional lymph node, consequently the vessels demonstrable by the presence of the india ink are only those which lie between the injected site and the node. An injected area of 3 cm. in diameter has been observed to drain into as many as seven of these vessels. The larger vessels run close to the skin for some distance before penetrating deeper into the subcutaneous tissue. During this part of the route they gradually converge, anastomosis is frequent, and finally a number of vessels pass together in the fascial planes separating muscles or portions of muscles to penetrate the capsule of the prescapular lymph node and enter the marginal sinus. The best demonstration of the lymph vessels between the skin and the node is obtained if the animal is killed within a few minutes of injection. These lymph vessels can also be demonstrated by injection of the skin after it and the underlying tissues have been removed from the dead animal.

Lymph reaching a node flows round the marginal sinus, being diverted at intervals into the lymph channels accompanying the trabeculae that leave the capsule at various points to traverse the cortex and form a fibrous network in the medulla. Maximow & Bloom (1942) have remarked on the strong development of the trabecular system of the lymph nodes of the ox. This is very apparent in the pre-

scapular lymph node. Staining of the nodular tissue following an intracutaneous injection of india ink is confined to a definite portion of the node. This stained portion is roughly cone-shaped, the base of the cone being the part of the marginal sinus served by the group of afferent vessels draining the injected skin site, and the apex of the cone being in the tissue of the medulla near the hilum where the lymph leaves the node by an efferent lymph vessel. One hundred prescapular lymph nodes have been examined following the intracutaneous injection of some detectable inoculum and in every case drainage has been to a portion, never to the whole, of the node.

*Demarcation of areas of the skin that drain to particular portions of the prescapular lymph node*

The possibility of associating certain areas of the skin with particular portions of the lymph node suggested itself when it was noted that injections made at a frequently used site, 20–25 cm. cranial to the point of the shoulder in the direction of the base of the ear, nearly always involved the same part of the node. In view of the generally accepted idea that the course of lymphatic vessels is subject to considerable variation, it is noteworthy that of forty-one injections made at this site thirty-eight caused involvement of the cranial aspect of the

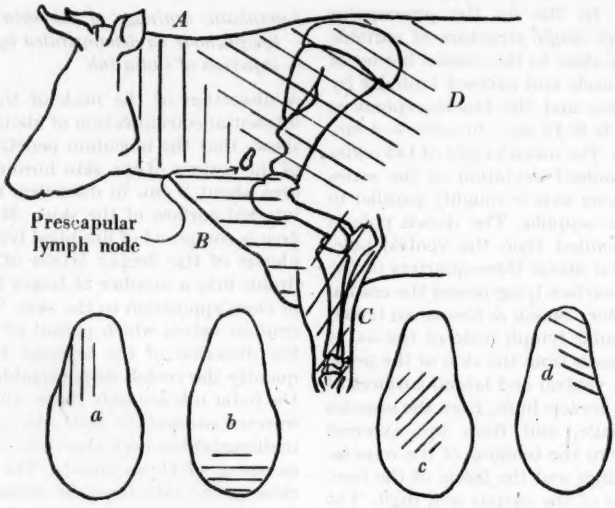



Fig. 1. Diagram showing areas of skin drainage, *A*, *B*, *C*, *D*, and associated portions of the prescapular lymph node, *a*, *b*, *c*, *d*.

The stained portion of the lymph node is limited in size even if the volume of the intracutaneous injection is increased, or if the interval between injection and examination is lengthened. The maximum area of the marginal sinus likely to be involved following one intracutaneous injection is about 12 sq.cm. It is evident that the strong development of the trabeculae, by hindering the lateral flow of lymph, confines the lymph from an afferent vessel to a portion of the marginal sinus, the cortex and the medulla. Although this has the effect of dividing the node into sections there is no watertight division as implied by Maximow & Bloom's statement (1942) that in the ox the trabecular system is so well developed that the nodules of the cortex are completely separated from one another.

dorsal half of the node while only three affected other portions.

Correlation of the position of the injected skin sites with involvement of particular portions of the node shows that the lymphatic drainage of the skin of the ox can be roughly divided into sections corresponding to particular portions of the prescapular lymph node (Fig. 1). In general, the drainage from each skin area is to the nearest portion of the node.

The skin areas and the corresponding portions of the node are defined in Table 1. As would be expected, the division between these sections is by no means distinct. An interesting demonstration of this can be got by injecting a detectable inoculum into the skin at a site on the mid-ventral line of the neck; on subsequent examination the inoculum is

usually apparent in the prescapular lymph node on each side of the neck.

*Involvement of more than one portion of the prescapular lymph node following an intracutaneous injection*

In eighty-seven of the 100 nodes examined the affected portion was confined to one area while in the remaining thirteen nodes more than one area was stained. When more than one area of the node is stained in this way the lymph from the injected site reaches the marginal sinus by at least two sets of afferent vessels, anastomosis having occurred with vessels draining another area of the skin. One factor that might influence the frequency of such an occurrence is the distance of the injection site from the node. The injection sites relating to the 100 nodes examined can be conveniently divided into two groups, fifty-eight injections were made within 25 cm. of the node and forty-two injections were made at a distance greater than 25 cm. from the node. It would seem reasonable to suggest that the farther the site of injection is from the node the greater would be the chance of anastomosis taking place but there is insufficient evidence to support this suggestion as multiple staining occurred in six out of the fifty-eight and in seven out of the forty-two nodes respectively.

Table 1. *Demarcation of areas of the skin of the ox associated with drainage to particular portions of the prescapular lymph node*

| Skin area | Portion of node involved |
|---|---|
| The cranial third of the neck and the segment of skin between that and the point of the shoulder | The cranial aspect of the lateral and medial surfaces of the dorsal half of the node, excluding the dorsal pole |
| The scapular region | The caudal aspect of the lateral and medial surfaces of the dorsal half of the node, including the dorsal pole |
| The ventral aspect of the neck from a point about 15 cm. caudal to the angle of the jaw to the brisket, extending dorsally to include the caudal portion of the jugular furrow and the point of the shoulder | The ventral pole |
| The forelimb from the distal end of the humerus to the carpus (no observations were made distal to the carpus) | The centre of the lateral surface |

The thirteen cases of multiple drainage can be classified into two groups, in seven instances the stained areas were close together within the bounds of the portion of the node associated with drainage from the particular skin area, and in the other six instances drainage was to quite separate portions of the node, one portion being that associated with drainage from the skin area in which the site was situated and the other portion being that associated with drainage from the adjacent skin area. Another factor, therefore, that might affect multiple drainage is the position of the injection site in relation to a zone of demarcation between different areas of drainage of the skin.

It can be concluded, therefore, that following an intracutaneous injection in the region under consideration the inoculum will drain to one particular portion of the prescapular lymph node except in a small proportion of cases when two or more parts of the node will be involved. These parts may be close together or far apart depending on the situation of the site of injection in relation to zones of demarcation between areas of skin drainage.

*Estimate of the number of afferent vessels supplying the prescapular lymph node of the ox*

Examination of many prescapular lymph nodes each having a small number of afferent vessels engorged with india ink or trypan blue solution makes it evident that the whole surface of the node, with the exception of the hilum, receives afferent vessels which in an engorged state have a diameter of approximately 0·5–0·75 mm. Many of these main vessels divide into two or three smaller branches just before penetrating the capsule to enter the marginal sinus. The vessels approach the node in groups, each group in its fascial plane which meets the surface of the node at an acute angle. Some of the vessels penetrate the capsule as soon as the node is reached, while others, before doing so, pass over the exterior of the capsule for a centimetre or more. Between forty and fifty vessels have been observed to supply an area of about 12 sq.cm. of the lateral surface. The size of the node is very variable but it is rarely less than 6 cm. long, 3 cm. broad and 1 cm. thick which gives a surface area of about 40 sq.cm. From this it is estimated that the total number of main afferent vessels supplying the node is between 150 and 200.

This estimate is based on incidental observations and may not approach the number that would be obtained if all the vessels could be counted, the impression being that 200 may be an underestimate for the prescapular lymph node of the ox.

## DISCUSSION

The fact that lymph entering the marginal sinus of the prescapular lymph node of the ox is confined to a portion of the cortex and the medulla is

considered worthy of comment especially when it is found that drainage from definite areas of the skin is associated with particular portions of the node. This restriction of drainage to comparatively small portions of the node appears to be greater than that found in some other species in which the trabecular system is not so well developed. For example, Nordmann (1928) states that, in man, lymph reaching a node may flow right round the marginal sinus to the hilum, and Drinker, Wislocki & Field (1933) and Drinker, Field & Ward (1934) found that injection with india ink of an afferent vessel of the popliteal lymph node of the dog caused staining of most of the node.

Consideration of the group of small prescapular lymph nodes in the horse with the one large node in the ox is of interest. The observations reported in this paper seem to indicate that the large node of the ox is simply a composite arrangement of the multiple nodes of the horse, division into small units being demonstrable within the structure of the large node as shown by the restriction of the drainage from different territories to particular portions. A study of the embryonic development of the prescapular lymph node of the ox might be of interest in this connexion.

A limited number of observations have been made on drainage from the skin to the prescapular lymph node in the goat. In this animal also, drainage from each skin site is confined to a portion of the node, but no attempt has been made to define areas of the skin associated with different portions of the node.

## SUMMARY

1. The intracutaneous injection of india ink and of trypan blue solution has been used to study the lymphatic drainage of the skin of the ox to the prescapular lymph node.

2. Drainage from each skin site is confined to a portion of the lymph node.

3. Areas of skin drainage have been defined associated with particular portions of the prescapular lymph node.

4. It is estimated that 150–200 afferent lymph vessels supply the prescapular lymph node of the ox.

5. The suggestion is made that the multiple arrangement of the prescapular lymph nodes of the horse has its counterpart in the functional division of the one large node of the ox into sections each dealing with a definable territory.

6. A limited number of observations suggests that the same arrangement exists in the goat.

The cattle used for these observations were healthy animals slaughtered for other reasons. I am indebted to Messrs W. J. Brownsea and R. H. Treadwell for technical assistance.

## REFERENCES

DRINKER, C. K., FIELD, M. E. & WARD, H. K. (1934). *J. Exp. Med.* **59**, 393.

DRINKER, C. K., WISLOCKI, G. B. & FIELD, M. E. (1933). *Anat. Rec.* **56**, 261.

HENDERSON, W. M. (1944). *J. Path. Bact.* **56**, 315.

MAXIMOW, A. A. & BLOOM, W. (1942). *A Textbook of Histology*, 4th ed. Philadelphia.

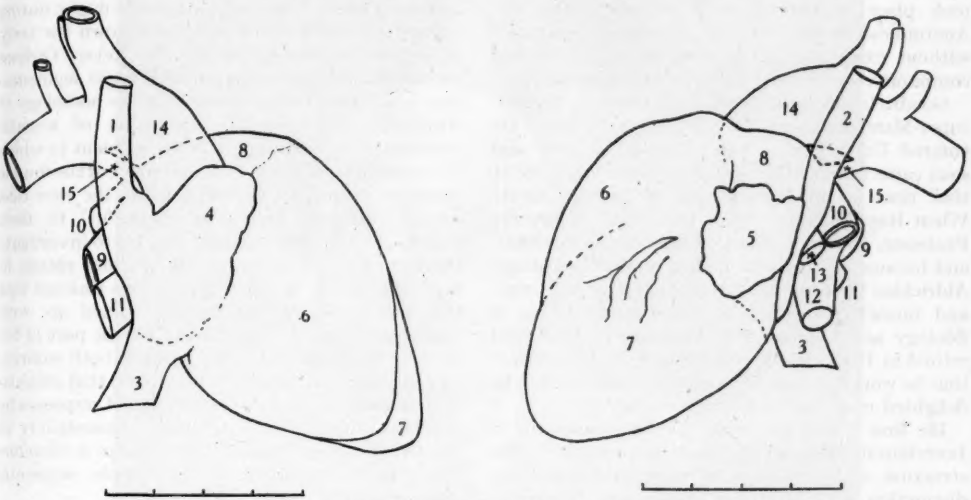
NORDMANN, M. (1928). *Virchows Arch.* **267**, 158.

SISSON, S. & GROSSMAN, J. D. (1938). *The Anatomy of the Domestic Animals*, 3rd ed. Philadelphia.

## ANATOMICAL NOTE

# BILATERAL ANTERIOR VENAE CAVAE IN A LION CUB

By K. J. FRANKLIN, *Nuffield Institute for Medical Research, Oxford*




Figs. 1, 2. Outline drawings, from right and left aspect respectively, of the heart and great vessels of a 3½ months old male lion cub. *1*, right anterior vena cava. *2*, left anterior vena cava. *3*, posterior vena cava. *4*, auricle of right atrium. *5*, auricle of left atrium. *6*, right ventricle. *7*, left ventricle. *8*, pulmonary trunk. *9*, right pulmonary artery. *10*, left pulmonary artery. *11*, pulmonary vein draining right apical and cardiac lobes. *12*, pulmonary vein draining right diaphragmatic, azygos and left diaphragmatic lobes. *13*, pulmonary vein draining fused left apical and cardiac lobes. *14*, aorta. *15*, closed ductus arteriosus. Scales in centimetres.

Bilateral anterior venae cavae are exceptional after a certain stage in the intra-uterine life of carnivora (Sonntag, 1921; Amoroso, Barclay, Franklin & Prichard, 1943). It is, therefore, of interest to record a second instance of their occurrence in *Felis leo*.

The cub in question was born on 8 July 1945 and died on 20 October 1945. The anterior venae cavae (Figs. 1, 2) were of approximately the same calibre, and no transverse anastomosis was discovered in the specimen (heart and lungs) as received from the Prosectorium of the Zoological Society of London. The ductus arteriosus was closed.

I wish to thank Colonel A. E. Hamerton, C.M.G., D.S.O., through whose courtesy I received the specimen, and Mr W. E. Lawrence, who removed and fixed it for me.

## REFERENCES

Amoroso, E. C., Barclay, A. E., Franklin, K. J. & Prichard, M. M. L. (1943). *Proc. Zool. Soc. Lond.* B, **113**, 43.
Sonntag, C. F. (1921). *Proc. Zool. Soc. Lond.* p. 587.

# IN MEMORIAM

## EDWIN STEPHEN GOODRICH

### 1868–1946

By the death of Edwin Stephen Goodrich, which took place at Oxford on 6 January 1946, the Anatomical Society has lost a member who can, without exaggeration, be claimed as the leading comparative anatomist in the world of his day.

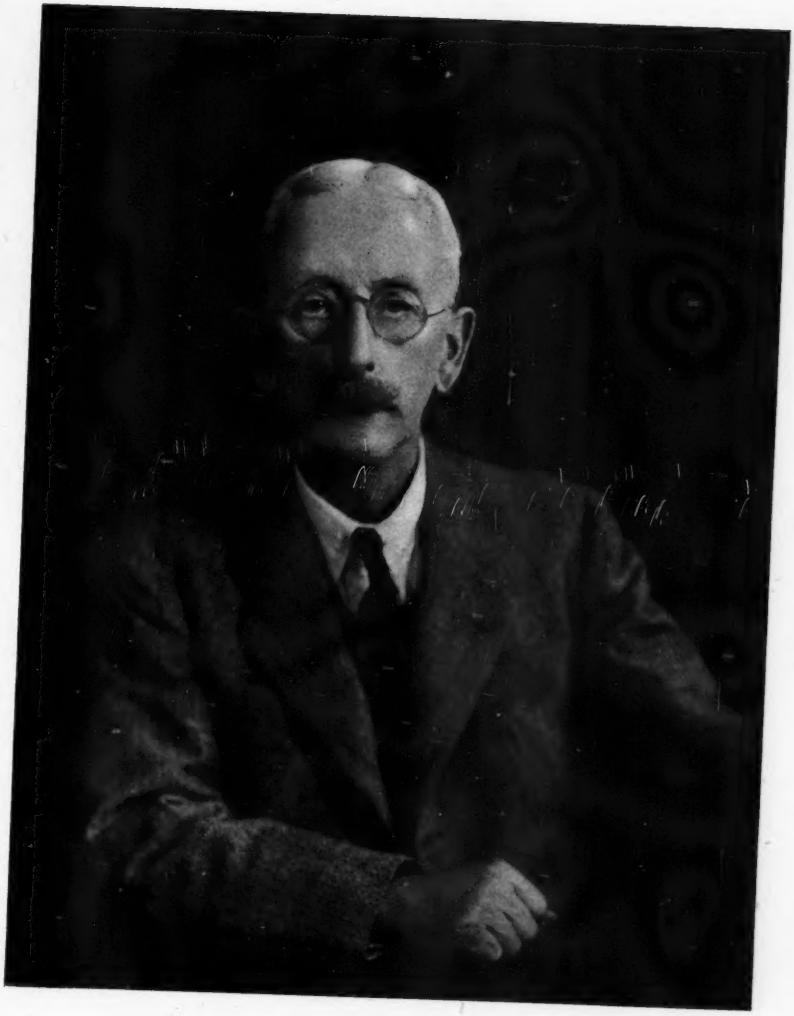
Goodrich was born on 21 June 1868, at Weston-super-Mare, and was brought up in France. He entered University College, London, in 1888 and soon came into contact with Ray Lankester, who at that time occupied the Chair of Zoology there. When Ray Lankester went to Oxford as Linacre Professor, Goodrich followed him as his assistant, and became successively Fellow of Merton College, Aldrichian Demonstrator in Comparative Anatomy, and himself succeeded to the Linacre Chair of Zoology and Comparative Anatomy in 1921. He retired in 1945. It may therefore be justly claimed that he worked all his life, and the work in which he delighted most was comparative anatomy.

He first made his name by his researches in Invertebrate Morphology, and particularly in the structure and development of nephridia and the distinction between these organs and the coelomoducts. This work had a profound effect on vertebrate morphology, however, because Goodrich was able to prove that since *Amphioxus* had true nephridia, homologous with those of annelid worms, the kidneys of vertebrates must be homologous with the genital ducts of those forms, and not with nephridia at all.

It was largely on the results of this work that Goodrich was elected into the Fellowship of the Royal Society in 1905 at the age of 36. At the same time he had already devoted his attention to vertebrate research, for one of his early papers dealt with the structure of the mesozoic mammalian jaws from Stonesfield. His next interest in vertebrates was concerned with the structure and morphology of cyclostomes and fishes, on which he contributed a volume in Lankester's series of text-books on Zoology. For this book, Goodrich undertook a series of researches of fundamental importance, as a result of which our knowledge of the most primitive living vertebrates was placed on a sound morphological basis. Goodrich studied the structure of the scales of fish and distinguished 'palaeoniscoid' from the 'lepidosteoid' type among what had previously been indiscriminately called 'ganoid' scales. This enabled living and fossil forms to be compared to greater advantage. He studied the development of the fins and this led him on to generalizations of great importance. By careful observation and acute reasoning he showed that a structure might during evolution become transposed up or down the body as a tune is transposed on the keys. Ordinal correspondence of position in metameric segmentation is not therefore necessary for the homology of structures. The orderly innervation of somatic muscle by the motor nerve of the segment to which it corresponds, in spite of displacement of the muscle rudiment during development, led to the view that somatic afferent nerves are 'faithful' to their muscles. From this, Goodrich was led to investigate the formation of the so-called brachial plexus in rays and skates, in which it had been claimed that the muscle derivatives became mixed up, with consequent lack of 'faithfulness' on the part of the nerves. He investigated the problem both embryologically and neurologically and found that stimulation of successive spinal nerves elicited responses by successive muscles and by fibres immediately in front of them and behind them. There is therefore only slight obscuring of the simple segmental arrangement.

Next, Goodrich attacked the problem of the segmentation of the vertebrate head, and showed that there is no evidence of the wholesale disappearance of segments and motor nerves in the occipital region, as envisaged by Fürbringer to make the occipital arches of different forms correspond in ordinal position. He devoted careful attention to the problem of the development of the auditory ossicles in reptiles, birds and mammals, and showed that Reichert's theory was the only one corresponding with the facts, and he discovered new facts which removed the last remaining obstacles to the theory.

His contributions to the *Journal of Anatomy* included a re-examination of the formation of the pericardio-peritoneal canals in dogfish, a study of the structure of the heart in reptiles, and an analysis of the autonomic nervous system into its morphological components of anterior nerve-root outflows and posterior nerve-root outflows. These studies culminated in his book on the *Structure and Development of Vertebrates* which is epoch-making, not only for the wealth of general principles and accurate details which it contains, but also because every sentence in it is based on personal research, and verification by him. Among many other items, it contains the best account of the development and evolution of the diaphragm.



EDWIN STEPHEN GOODRICH
1868–1946

UNIV. OF MICH.

Goodrich's last contribution to the *Journal of Anatomy* was a reinvestigation of his own suggestion that the hooked shape of the 5th metatarsal in reptiles was a characteristic feature of the Sauropsida.

Throughout Goodrich's work there is to be found the theme of the significance of morphological characters for the elucidation of the phylogenetic history of their possessors. While primarily a comparative anatomist, he always maintained the most lively interest in the progress of experimental embryology and of genetics, with the result that he was, with his unequalled knowledge of the structure and development of the most diverse forms, in a unique position to appreciate the problems of general importance to the whole of biology. His analysis of the relations of genetics to embryology in the interpretation of evolution has provided the general line of thought on which any progress must be based.

In addition to his distinction as a zoologist, Goodrich was a consummate artist, and he used his talent not only to paint water-colour pictures of landscapes which are a delight to look at, but also to illustrate his lectures with chalk on the blackboard and his papers with text- and plate-figures of unrivalled clarity and beauty.

Of a very reserved and retiring nature, his modesty was almost unbelievable. Beneath it was a very human and friendly personality, quick to see and appreciate a joke, helpful and intensely loyal.

In 1913 he married Dr Helen Pixell with whom he collaborated in researches on Protozoa. The Anatomical Society will wish to associate itself with her in her bereavement which is also their very grievous loss.

G. R. DE BEER

## PROFESSOR J. FLORIAN

We have learned with deep regret, through the columns of *Nature* of 2 March, of the death in May 1942, of Professor J. Florian of the Masaryk University, Brno. Professor Florian was a valued contributor to the *Journal of Anatomy* between 1930 and 1935 at which time he worked with Professor J. P. Hill at University College, London. With him he contributed also to the *Philosophical Transactions of the Royal Society* an account of the 'Dobbin' embryo. Professor Florian was a welcome visitor at meetings of the Anatomical Society, where he had many friends. We mourn the tragic circumstances of his loss.